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# JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

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# PREDATOR INFLUENCE ON SHORE-LEVEL SIZE GRADIENTS IN *TEGULA FUNEBRALIS* (A. Adams)

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**Abstract:** Reproductive portions of *Tegula funebralis* (A. Adams) populations are shown to be immune to seastar predation through a combination of predator preference for larger snails and a withdrawal behavior that favors the escape of smaller snails after capture by a seastar. Experimental addition of *Pisaster ochraceus* (Brandt) in winter causes changes in the intertidal distribution of *Tegula funebralis* similar to those observed during the summer increase in seastar numbers. It is suggested that these results supplant the hypothesis that lowered prereproductive mortality influences formation and maintenance of vertical size gradients in the lower intertidal zone.

## INTRODUCTION

Many intertidal gastropods have a vertical size gradient in the range of their distribution. This distribution was originally thought to result from gradients in juvenile mortality in intertidal regions (Vermeij, 1972), but Bertness (1977) suggested that generalizations about specific causative factors cannot adequately explain the development of size gradients since they might result from evolutionary optimization due to several interacting factors.

Larger *Tegula funebralis* are distributed lower in the intertidal zone than the more abundant smaller snails (Paine, 1969). For the larger snails, Paine (1969) suggested that the advantages of increased individual fecundity due to lowered density and greater food availability outweigh the disadvantages of habitat overlap with the predatory seastar, *Pisaster ochraceus*, in the lower intertidal zone. Paine (1969, 1971) demonstrated the effects of physiological stress on larger snails in the upper intertidal zone, but he did not account for the maintenance of high densities of smaller snails in the upper area, nor did he feel that *Tegula funebralis* generally has adequate escape responses against *Pisaster ochraceus* predation.

Obrebski (pers. comm.) determined that lower intertidal *Tegula funebralis* migrate upwards concurrently with seasonal increases in seastar abundance. He also recorded associated fluctuations in snail size and argues that lower escape velocities of smaller snails tend to substantiate Vermeij's (1972) hypothesis. The present study is a more detailed examination of *T. funebralis* (A. Adams) migration at a different locality. New information on seastar feeding behavior and a snail escape mechanism is reported which suggests an alternative explanation of *T. funebralis* size

gradients. In particular, smaller snails are shown to be immune from digestion by *Pisaster ochraceus* (Brandt).

## Field Sampling Methods

Field sampling was done at the Point Reyes National Seashore, California, at the Palomarin Beach access (122°45′30″ W; and 37°56′ N). The area was chosen because of the abundance of *Tegula funebralis* and *Pisaster ochraceus*, the flat topography, the isolation from human disturbance, and the absence of significant numbers of prey, other than *Tegula*, for feeding seastars.

A transect measuring 10 × 50 m with the long axis perpendicular to the water was established. The vertical difference between the upper and lower intertidal levels of the transect was <2 m. The entire transect was exposed at a 0.0 tide, except for a few shallow pools. Five 10 × 10 m squares were labeled X, A, B, C, and D from the seaward edge upward. The lowest square (X) was only sparsely populated with *Tegula funebralis*.

Samples were taken between March 1978 and January 1979 to determine *Pisaster ochraceus* abundance and *Tegula funebralis* size and abundance. All seastars were counted at each sampling date by thoroughly examining the transect. Snails were counted in 10 randomly chosen 625-$m^2$ quadrats in each of Squares A, B, C, and D at each sampling date. From 2 to 10 of these quadrats were also used to measure snail shell size, depending upon the abundance of snails.

Shell size was measured with vernier calipers to the nearest 0.1 mm using the method of Paine (1969). All data were collected in the field and no snails or seastars were ever removed from the transect.

Size and abundance data were analyzed using a nested analysis of variance (ANOVA) design. Abundance data were compared in a two-level analysis with the highest treatment level as sampling dates, then squares within dates, and finally quadrats within squares as the lowest level. Snail shell size data were taken in a three-level design with levels from highest to lowest: sampling dates, squares within dates, quadrats within squares, and shell sizes of snails within quadrats. The computations were performed with the aid of a computer program adapted from Sokal & Rohlf (1969).

## Results

### *Pisaster ochraceus* abundance

Seastars increase in abundance and maximal tidal level to a peak in mid-summer and decline thereafter (Fig. 1).

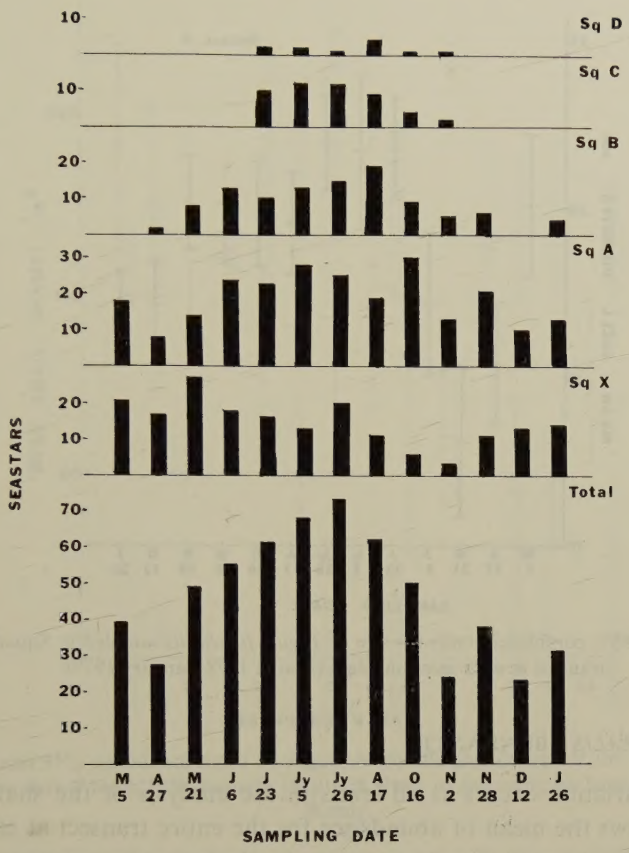


Fig. 1. Numbers of *Pisaster ochraceus* found in the five squares of the field transect at each sampling date (March 1978–January 1979).

*TEGULA FUNEBRALIS* SHELL SIZE

Snail shell size data for the entire transect show no significant variation between months, but there is highly significant variation in the lower levels of the analysis. Further analysis of the individual squares in a two-level design shows a significant variation ($P < 0.05$) between months for sizes of snails found in the lowest square sampled (Square A). Fig. 2 shows that the mean size of snails in Square A reaches a maximum in mid-summer and declines through the fall. The correlation between the number of seastars and the size of snails in each square was positive and significant ($r = 0.23$, $n = 1230$, $P < 0.001$). The correlation between size of snails and tidal level (squares) was negative and significant ($r = -0.23$, $n = 887$, $P < 0.001$).

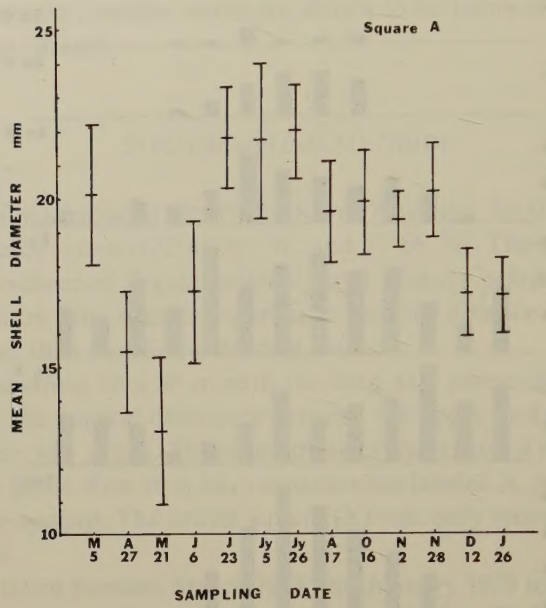


Fig. 2. Means and 95% confidence limits for size of *Tegula funebralis* sampled in Square A of the field transect at each sampling date (March 1978–January 1979).

### *TEGULA FUNEBRALIS* ABUNDANCE

Significant variation occurs at all levels in the analysis of the snail abundance data. Fig. 3 shows the mean of abundance for the entire transect at each sampling date. Fig. 4 depicts the snail abundance means for the lowest square measured (Square A). The graphs show the decline in density through the early summer months to a mid-summer low and slight recovery in numbers through the fall. The correlation between seastar numbers per square and snail abundance within squares was negative and significant ($r = -0.203$, $n = 450$, $P < 0.001$).

### EXPERIMENTAL INCREASE OF *PISASTER OCHRACEUS* ABUNDANCE IN WINTER

Two transects were established at the Palomarin site. One was the seaward 30 m of the previously described (see p. 2) transect (Transect 1). A second transect (Transect 2) was established in a comparable area 50 m to the north.

Both transects were sampled monthly for the 2 months prior to experimental manipulation in February and March. Sampling was performed as described above (p. 2). Seastars were counted in each of the three 10 × 10 m squares of each transect as well as in the area above the transects. There were no seastars in the higher areas at these times. *Tegula funebralis* were subsampled for shell size and abundance in Squares A and B of the transects. Ten 625-cm$^2$ quadrats were randomly selected and all snails found in each quadrat were counted and measured.

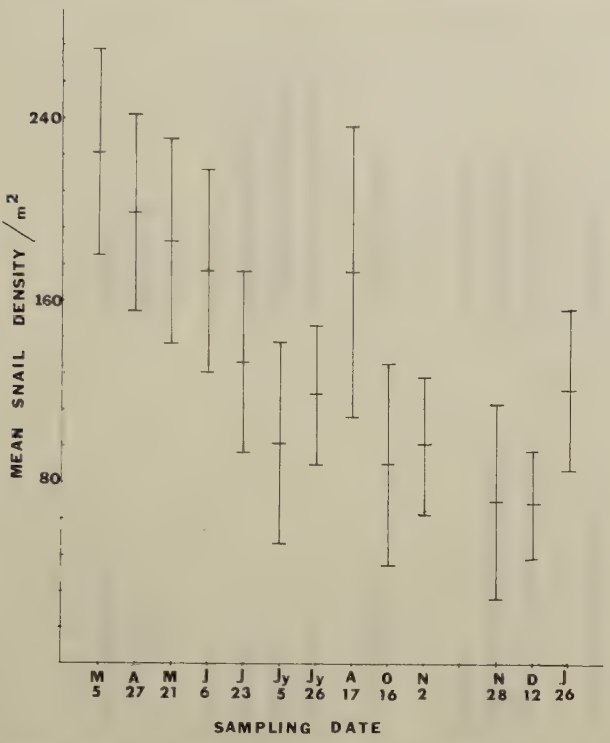


Fig. 3. Means and 95% confidence limits of *Tegula funebralis* abundance for the entire field transect at each sampling date (March 1978–January 1979): 28 Nov.–26 Jan. samples based on Squares A and B only.

On 22 February another sample was taken. At this time laboratory-maintained specimens of *Pisaster ochraceus* were brought to the field site. Seastars were added to Transect 1 until the total numbers in each of the squares was comparable to peak summer abundance. Transect 2 was used as a control area. Seastar abundance was checked and augmented as necessary on the 3 following days. Snail size and abundance were sampled 2 and 5 days after the transplant in each transect.

On 23 March the above experiment was repeated using Transect 1 as a control and adding seastars to Transect 2. Snail size and abundance was sampled 2 and 4 days after the transplant.

The sampling of snail shell size was in a three-level nested ANOVA design with levels: among sample dates, between squares within dates, between quadrats within squares, and snails within quadrats. Snail abundance was in a two-level design with levels as above minus the lowest level.

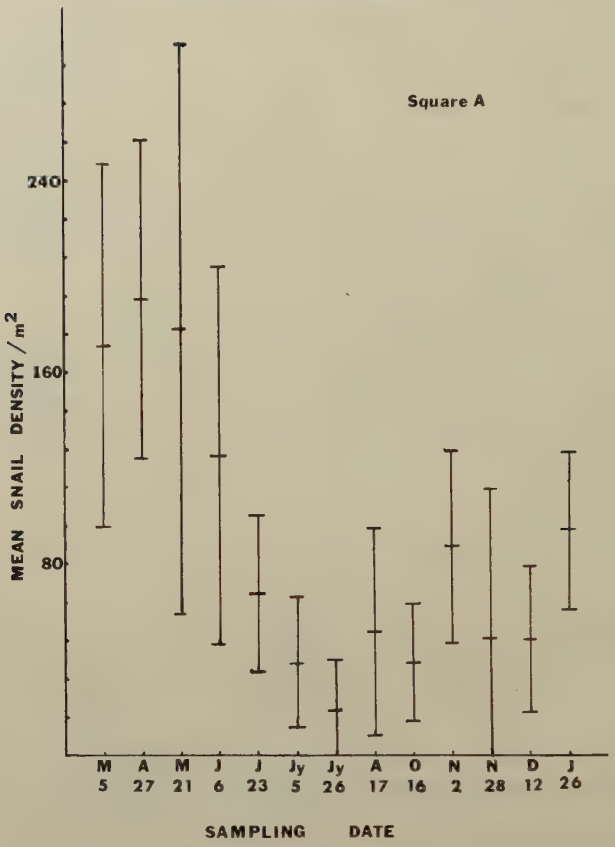


Fig. 4. Means and 95% confidence limits for *Tegula funebralis* abundance in Square A of the transect at each sampling date (March 1978–January 1979).

*Seastar abundance during field experiments*

Fig. 5 shows the number of seastars present in Square A of each transect for the duration of the two field experiments, as well as data from the two previous months.

Tegula funebralis *abundance during field experiments*

There was no significant change in density in either transect during the period of either experiment. Abundance was comparable in both transects.

Tegula funebralis *size during field experiments*

The increase in size in Square A of each transect after experimental manipulation (Fig. 6) was significant ($P < 0.05$). There was no significant change in size of snails

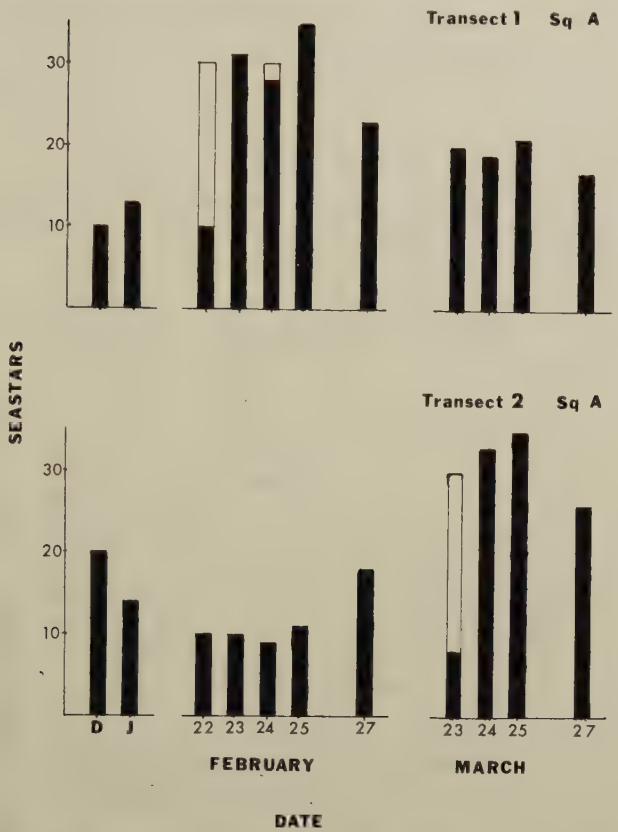


Fig. 5. Seastar numbers during field experiments: solid bars indicate number present; clear bars show those additional seastars added on that sampling date.

when each transect was measured as a control (Fig. 6). Square B of both transects showed no significant changes.

## SIZE OF *TEGULA FUNEBRALIS* PREFERRED BY *PISASTER OCHRACEUS*

The size of snail that is actually consumed by foraging seastars is a key factor in understanding the dynamics of the snail population. Paine (1969) estimated the number of *T. funebralis* per unit area consumed by seastars, but did not include specific data on the size range preferred by the seastar. Studies to determine the seastars' preference in snail size are described below.

Snail shells held by seastars in the feeding position were measured in July 1978. All feeding seastars encountered in a wide portion of the area were used. In addition, six similarly-sized seastars were placed, three in each half of a divided water table with a screened top. Twenty snails, five each of four size classes were placed in each half. At the end of each 24-h period, any consumed snails were removed, measured,

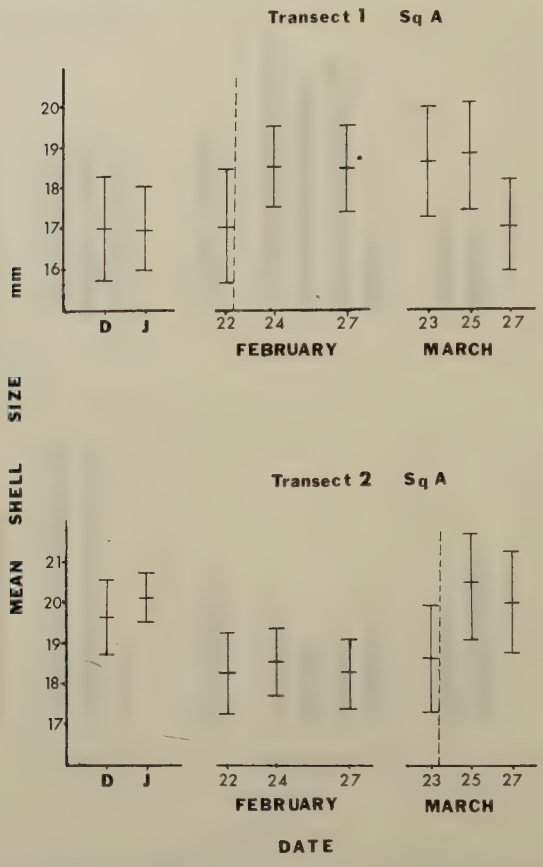


Fig. 6. Means and 95% confidence limits of *Tegula funebralis* shell size found in Square A of the transects during the two field experiments: dotted lines show times of experimental increase in seastar numbers.

TABLE I

Experimental data for *Tegula funebralis* size preferred in laboratory by feeding *Pisaster ochraceus*.

| Shell size class | 1 8–12.4 mm | 2 12.5–16.9 mm | 3 17–21.4 mm | 4 21.5–26 mm |
|---|---|---|---|---|
| Tank A | | | | |
| Mean number eaten per day | 0.12 | 0.65 | 0.82 | 1.0 |
| Mean % survival per day | 97.65 | 87.05 | 83.5 | 80 |
| Tank B | | | | |
| Mean number eaten per day | 0.05 | 0.17 | 0.65 | 0.76 |
| Mean % survival per day | 98.8 | 96.5 | 87.05 | 84.7 |

and replaced with snails of the same size class. The design was analysed with a two-level ANOVA with levels: among replicates, size classes within replicates, and daily survival within size classes. Data were collected for 17 days.

*Size preference*

The data (Table I, Fig. 7) show that seastars prefer the larger-sized snails. Table I shows a significantly ($P < 0.001$) higher survivorship in the smaller-size classes.

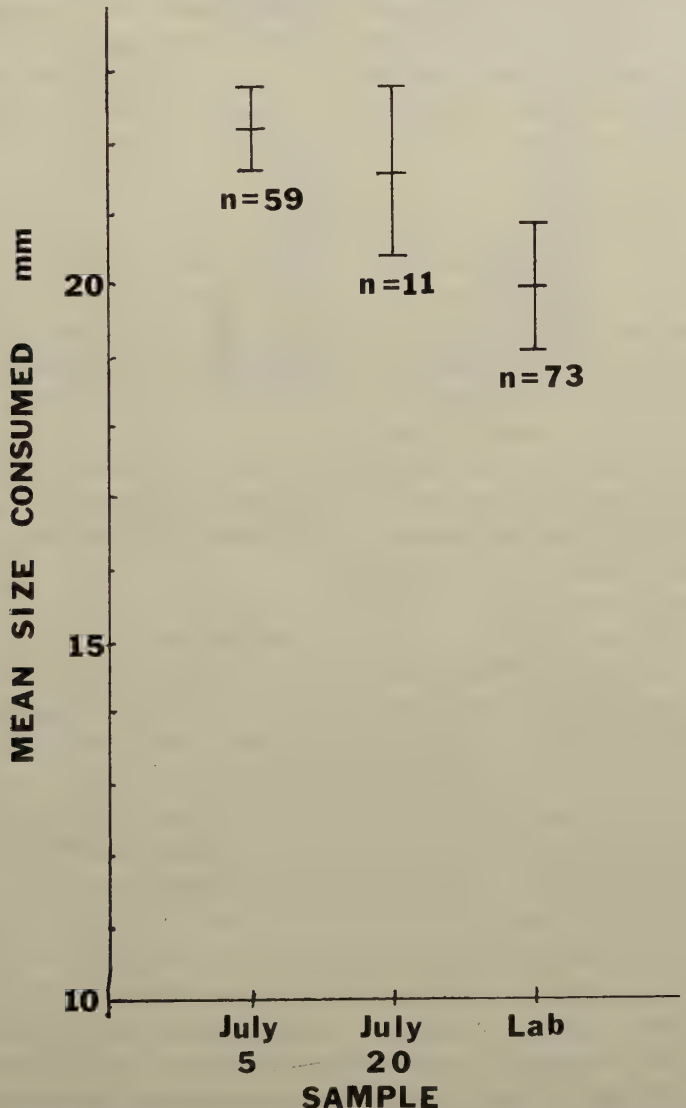


Fig. 7. Means and 95% confidence limits of snail sizes consumed by *Pisaster ochraceus* in two field and one laboratory samples.

The mean sizes of snails eaten in the field are compared to those found in the laboratory in Fig. 7. It is apparent that larger-sized snails are consumed in the field. The difference between laboratory and field results is probably an artifact of the laboratory conditions. Comparison of the field data to the snail sizes found in the transect (see p. 4) shows that the sizes consumed are greater than most average sizes found in the field. The field samples were taken when the seastars were spread through their peak range of tidal height, and, thus, they encountered snail populations with a lower mean size than those found in the lowest square (A) of the transect.

Visual observation of the laboratory predation experiments showed many instances of a seastar releasing unharmed small snails after capture. Also a greater proportion of small snails was found in a withdrawn position indicating success of the escape response as described in the next section.

## STUDIES ON NEW ASPECTS OF ESCAPE BEHAVIOR

During laboratory observations of *Pisaster ochraceus* predation on *Tegula funebralis*, it was discovered that the snails withdrew their entire body and operculum into the shell past the first third of the body whorl, following capture by a seastar. This behavior was observed when snails that appeared to have been eaten during feeding experiments were subsequently found to be alive. The following experiment was performed to test the effectiveness of this escape response.

The first quarter of the body whorl was removed by grinding as a treatment to eliminate its protection. Snails were classed by size and marked before grinding and used a day or more after the operation. No difference in escape velocity was found in snails tested before and after grinding.

Two seastars were placed in each of four 10-gal glass aquaria with running sea water and screened tops. Seastars were randomly selected from a group of roughly equivalent wet weights and sizes. Each tank was supplied with 20 snails, five each of four size classes. Two tanks held normal snails and the other two held treated snails. All tanks were checked daily for 7 days, and consumed snails were scored and replaced by live snails of the same size class and type.

The experiment was designed for analysis with a three-level ANOVA. The levels from highest to lowest were: among treatments, replicates within treatments, size classes within replicates, and survival within size classes.

### *Effectiveness of withdrawal behavior*

Mean percent survival for all size classes of treated snails was 76.07, 10% lower than the 86.07% survival of controls. This difference was significant ($P < 0.05$) while there was no significant difference between replicates within treatments. Fig. 8 presents the number of snails consumed in each treatment. The difference between size classes eaten is similar to the data presented earlier and is also significant ($P < 0.001$).

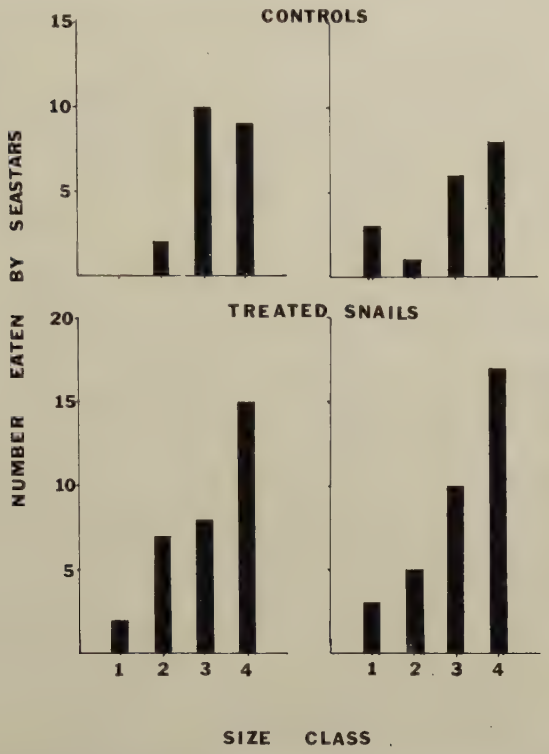


Fig. 8. Numbers of *Tegula funebralis* eaten by seastars in experiment to test effectivness of withdrawal response: size classes are the same as those listed in Table I.

## Discussion

Limits on lower distribution of intertidal animals set by predation are widely reported (Connell, 1970; Paine, 1976; Bros, 1978). The present study emphasizes behavioral aspects of a predator and the behavioral and morphological aspects of a mobile prey that allows the latter to extend their range into that of the former. Field data from the Point Reyes area agree with the observations by Obrebski (pers. comm.) indicating that a seasonal change in *T. funebralis* distribution occurs concurrently with the upward migration of *Pisaster ochraceus* and a change in snail density in the lower intertidal zone. This correlation of the influence of seastars on turban snail migration in two localities is further supported by the field and laboratory experiments.

The field experiments demonstrated that *P. ochraceus* has a measurable short-term effect on *Tegula funebralis* size distribution. The seastars prefer the larger snails. While larger snails have faster escape speeds (Obrebski, pers. comm.), the smaller snails, through a combination of behavioral and morphological charac-

teristics, escape predation by *Pisaster ochraceus*. Previous natural history studies (Mauzey, 1966; Landenberger, 1967, 1968; Dayton, 1971; Jillson, 1973; Paine, 1976) generally assume that escape from predation occurs only by having a larger size or by migrating into areas inaccessible to the predators. The fact that all *Tegula funebralis* show a "running" escape response (Feder, 1963; Yarnall, 1964), and smaller snails may be only rarely consumed by seastars, suggests an alternative explanation for the observed gradients and migration in *T. funebralis*. Physiological factors (Paine, 1971) cause larger snails to migrate lower where they can be better maintained due to greater abundance of food resources. Due to increasing numbers of encounters with seastars, some larger snails may be eaten and smaller snails move upward resulting in the observed increase in average snail size and the corresponding decrease in snail abundance during upward seastar migration. The reproductive characteristics of *T. funebralis* provide a mechanism for the maintenance of these distributional patterns despite a large snail overlap with the predator. Paine (1971) showed that *T. funebralis* are fully mature at sizes >18 mm, well below the seastars' preferred prey size of 20–23 mm. Thus, a proportion of the population that is reproductive is relatively immune from seastar predation. The tendency for smaller snails to migrate into the upper intertidal zone due to to their escape response to seastars does not explain the selective mechanisms actually maintaining small snails in the upper intertidal zone, since they are often able to escape digestion when caught. Other predators eat snails. Smaller *Tegula* are more susceptible to predation by crabs than large ones (H. Boley, pers. comm.). It is possible that the tendency for small snails to migrate upwards due to the presence of seastars is maintained because it aids in their removal from the range of activity of other predators to which they have less effective escape responses and with which seastar distributions are correlated.

Fawcett (1979) found variations in intensity and type (seastar, crab, octopus) of predation on *T. funebralis* which resulted in alterations of the intertidal distribution of snails at different latitudes. His results support the present discussion.

Experimental manipulation of seastar populations in other localities should provide further corroborative evidence for the foregoing hypothesis and help to define the effects of other changes as possible causative factors in *T. funebralis* migration. Further experimentation is also needed to verify assumptions about the effects of other predators.

As suggested above, a complex system of interactions results in a size gradient in the vertical distribution of *T. funebralis* intertidal populations. These facts support further a detailed evolutionary explanation as suggested by Bertness (1977). The simpler explanation of lowered post-larval prereproductive mortality in higher areas suggested by Vermeij (1972) is considered insufficient. Rather, a lowered predation effect on part of the reproductive portion of the snail population provides an explanation more congruent with the new data and natural history information presented here.

## Acknowledgement

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# MORTALITY ESTIMATES FOR *PENAEUS VANNAMEI* Boone IN A MEXICAN COASTAL LAGOON

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**Abstract:** Marked and unmarked shrimp were maintained in enclosures in order to estimate mortality rates. Regular sampling and measurement of shrimp in small enclosures (10 $m^2$) resulted in higher mean mortality rates for tagged shrimp (34% per wk) than for untagged shrimp (12% per wk). These estimates include death due to handling in addition to natural mortality. Laboratory tests also indicated higher mortality rates in tagged shrimp. In larger enclosures (2100 $m^2$) where sampling was restricted, lower rates were obtained for tagged shrimp (17% per wk) even though predators were present. Freshly tagged shrimp were found to be vulnerable to sudden changes in environmental conditions.

A tag-release experiment indicated a maximum natural mortality rate of 31% per wk in the open lagoon. This is a high estimate, and the natural mortality rate of untagged shrimp in the lagoon probably lies at the lower end of the range 12–31% per wk.

## INTRODUCTION

The geography and general ecology of the Caimanero–Huizache lagoon system have been described by several authors (Edwards, 1977, 1978; Warburton, 1978). The lagoon lies on the Pacific coast of Mexico 25 km south of the city of Mazatlan (Fig. 1). The commercial fishery has been described by Edwards & Bowers (1974). Four species of shrimp are caught: *Penaeus vannamei* Boone and *P. stylirostris* Stimpson (subgenus Litopenaeus), *P. brevirostris* Kingsley and *P. californiensis* (Holmes) (subgenus Farfantepenaeus). Of these *P. vannamei* constitutes ≈90% of the total lagoon catch (Sepulveda, 1976). Aspects of the biology of this species have been studied by Chapa & Soto (1969), Edwards (1977), and Menz & Bowers (in prep.). Few attempts have been made to estimate mortality rates of shrimp from the lagoon. Sepulveda (1976) calculated total mortality coefficients from commercial catch data, and Edwards (1977) made an estimate of natural mortality rates for *P. vannamei* in a single enclosure experiment. The majority of mortality estimates made for penaeids in commercial fisheries relate to work in the open sea (Klima, 1963; Kutkuhn, 1966). Estimates of mortality for *P. duodarum* and *P. aztecus* in enclosed bays off the coast of North Carolina, U.S.A. have also been made (McCoy, 1968; Purvis & McCoy, 1974).

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The object of the present study was to obtain natural mortality estimates from a variety of experimental approaches using both tagged and untagged shrimp. This was considered to be of importance in evaluation of the possible impact of predation on the commercial fishery.

## Materials and Methods

Two sizes of enclosure were used. A corral 30 × 70 m was employed in September 1978, and smaller enclosures 5 × 2 m were used for a series of experiments between October 1978 and March 1979. Both types of enclosure were of galvanized wire mesh 0.5 $cm^2$, standing 93 cm high. The wire was supported by wooden stakes driven into a fine mud substratum. The situation of the enclosures is shown in Fig. 1.

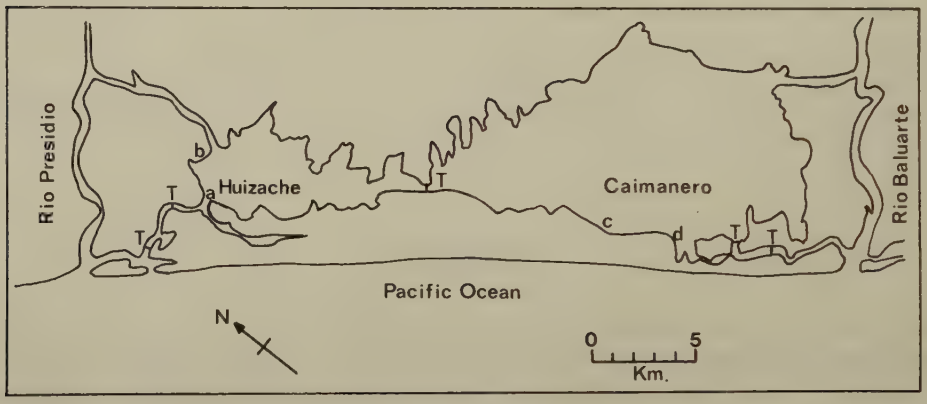


Fig. 1. The Caimanero-Huizache lagoon system showing sites of enclosures: a, Huizache small enclosures; b, Huizache culture pond; c, Caimanero small enclosures; d, large corral; T, tapos.

Estimates of mortality were also obtained from a tagged population of known size released into a disused culture pond normally open to the Huizache lagoon. This pond (20,625 $m^2$) was sealed from the open lagoon by wire mesh for 17 days, after which it was re-opened to permit passage of tagged shrimp into the lagoon. Subsequent re-captures by fishermen were monitored, and returns were encouraged by the offer of a reward of ≈U.S. $1.00 per tag with whole shrimp.

Shrimp for enclosure studies were normally obtained by local cast-net from the adjacent lagoon or estero. Shrimp caught by beam trawl were used in the large corral experiment of 6 September 1978. Freshly caught shrimp were used except where specified (see Table I).

In the large corral and culture pond, sampling was carried out by cast-net. In the smaller enclosures, a combination of hand-net and hand-spread cast-net was used. It was necessary to disturb buried shrimp by hand from the perimeter of the small

TABLE I

Summary of mortality estimates from enclosure experiments: mean instantaneous mortality rate for small enclosure tagged shrimp 0.059 ($s = 0.0264$), equivalent to 34% per wk ±10% (95% confidence limits); mean instantaneous mortality rate for untagged shrimp 0.018 ($s = 0.0107$), equivalent to 12% per wk ±4% (95% confidence limits); shrimp were fresh caught with the exception of Experiments 6 and 20 which involved survivors from Experiments 14 and 15; *, results not used in calculations of instantaneous mortality rates.

| Expt. no. | Site | Salinity (‰) | | Temp. (°C) | | Days run | Shrimp category | No. at start | No. at finish | Mean size | Instantaneous mortality rate | Correlation coefficient | % per wk | Date |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Start | Finish | Start | Finish | | | | | | | | | |
| 1 | Huizache | 10 | – | 25 | 24 | 16 | Floy tag | 29 | 12 | 24.6 | 0.062 | 0.987 | 35 | 15 Nov. 1978 |
| 2 | Caimanero | 32 | 40 | 25 | 24 | 43 | Floy tag | 14 | 5 | 26.0 | 0.024 | 0.980 | 15 | 6 Dec. 1978 |
| 3 | Huizache | 32 | 34 | 24 | 24 | 40 | Floy tag + cotton | 19 | 2 | 19.3 | 0.057 | 0.991 | 33 | 11 Jan. 1979 |
| 4 | Huizache | 34 | 27 | 25 | 23 | 5 | Floy tag + cotton | 28 | 0 | 18.1 | 0.650 | – | 99 | 24 Jan. 1979* |
| 5 | Huizache | 13 | 34 | 19 | 24 | 15 | Floy tag + cotton | 20 | 2 | 18.3 | 0.157 | 0.963 | 67 | 2 Feb. 1979* |
| 6 | Caimanero | 39 | 44 | 24 | 26 | 32 | Floy tag + cotton | 29 | 10 | 23.6 | – | – | – | 7 Feb. 1979* |
| 7 | Huizache | 32 | 34 | 25 | 24 | 30 | Floy tag + cotton | 15 | 2 | 17.9 | 0.069 | 0.972 | 37 | 14 Feb. 1979 |
| 8 | Huizache | 32 | 34 | 24 | 24 | 28 | Floy tag + cotton | 17 | 1 | 19.1 | 0.102 | 0.988 | 51 | 16 Feb. 1979 |
| 9 | Huizache | 32 | 34 | 24 | 24 | 19 | Floy tag + cotton | 7 | 2 | 19.7 | 0.069 | 0.994 | 37 | 1 Mar. 1979 |
| 10 | Huizache | 30 | 34 | 21 | 24 | 29 | Floy tag + cotton | 8 | 3 | 17.4 | 0.029 | 0.982 | 18 | 19 Feb. 1979 |
| 11 | Caimanero | 26 | 30 | 28 | 29 | 12 | Unmarked | 37 | 24 | 20.0 | 0.039 | 0.949 | 24 | 27 Oct. 1978 |
| 12 | Caimanero | 32 | 40 | 25 | 24 | 42 | Unmarked | 15 | 10 | 17.0 | 0.010 | 0.962 | 7 | 6 Dec. 1978 |
| 13 | Huizache | – | – | – | – | 21 | Unmarked | 40 | 34 | 19.0 | 0.007 | 0.782 | 5 | 13 Dec. 1978 |
| 14 | Caimanero | 35 | 23 | 25 | 21 | 39 | Unmarked | 50 | 40 | 15.8 | 0.006 | 0.988 | 4 | 19 Dec. 1978 |
| 15 | Caimanero | 35 | 23 | 25 | 21 | 39 | Unmarked | 100 | 71 | 17.0 | 0.008 | 0.919 | 6 | 19 Dec. 1978 |
| 16 | Caimanero | 39 | 44 | 24 | 26 | 16 | Cotton banded | 17 | 10 | 22.3 | 0.034 | 0.981 | 21 | 12 Feb. 1979 |
| 17 | Caimanero | 41 | 44 | 29 | 26 | 8 | Cotton banded | 36 | 23 | 24.2 | 0.063 | 0.993 | 36 | 2 Mar. 1979* |
| 18 | Huizache | 32 | 32 | 24 | 25 | 35 | Cotton banded | 12 | 6 | 19.2 | 0.023 | 0.980 | 15 | 11 Jan. 1979 |
| 19 | Huizache | 34 | 32 | 25 | 24 | 24 | Cotton banded | 20 | 15 | 17.7 | 0.013 | 0.995 | 9 | 24 Jan. 1979 |
| 20 | Caimanero | 30 | 44 | 19 | 27 | 35 | Cotton banded | 40 | 18 | 22.9 | 0.023 | 0.969 | 15 | 31 Jan. 1979 |
| 21 | Caimanero | 32 | 40 | 25 | 24 | 42 | Uropod clipped | 15 | 8 | 17.7 | 0.013 | 0.990 | 9 | 6 Dec. 1978 |
| 22 | Caimanero | 32 | 40 | 25 | 24 | 42 | Uropod clipped | 15 | 6 | 25.8 | 0.021 | 0.984 | 13 | 6 Dec. 1978 |
| 23 | Caimanero | 32 | 40 | 25 | 24 | 42 | Unmarked | 11 | 4 | 24.7 | 0.024 | 0.990 | 15 | 6 Dec. 1978 |

enclosures. Fishing in small enclosures was normally continued until no further shrimp were caught. Sampling frequency varied with each experiment.

Four categories of shrimp were used for stocking: (1) untagged shrimp; (2) untagged shrimp with 3-4 mm clipped from one uropod; (3) untagged shrimp with a coloured band of cotton thread tied around the articulation between the sixth abdominal segment and the uropod–telson complex; (4) shrimp tagged with Floy streamer tags with individual numbers (Floy Tag Co. FTSL-73 vinyl tag). The tag was inserted between the first and second abdominal segments (Marullo *et al.*, 1976). With the exception of specified experiments (see Table I), these shrimp also had a cotton band. The object of this band was the recording of individual moult patterns in conjunction with contemporary growth studies. Size was measured as carapace length to the nearest 0.1 mm using dial calipers.

Laboratory tests were also made to assess the effect of tagging on mortality rates. Shrimp were caught by cast-net and transported to the laboratory in aerated plastic containers. They were normally left overnight in aquaria to recuperate, and tagged and measured the next morning. Checks were carried out at intervals throughout the day to record deaths. Marked and unmarked shrimp were mixed in the aquaria.

## Results

Mortality estimates are summarized in Tables I and II. For the small enclosure experiments (Expt. 1 to 23) $\log_e$ number of shrimp in the enclosure was plotted against time in days, and a regression line fitted by the method of least squares. The slope of each line is the daily instantaneous coefficient of total mortality ($Z$) operating during the experiment. The equivalent weekly percentage mortality is given in Table I.

In the large enclosure and culture pond experiments (Expt. 24–26), total numbers of shrimp could not be determined and the mortality rates were calculated from catch per unit effort. The unit of effort employed was the given number of throws of a standard cast-net. The actual number of throws varied from 13–45, and the mortality rates were calculated from the plot of $\log_e$ mean catch per throw for each sampling day against time in days. In the culture pond experiment the initial tagged population was enclosed for 17 days prior to release. During this period regular sampling permitted an estimate of the daily instantaneous total mortality coefficient (Table II, Expt. 26), and this was used to predict the probable number released into the open lagoon on Day 18 (Table II, Expt. 27). The difference between this number (975) and the total number recaptured by fishermen in 21 days (324) was used to estimate a maximum possible mortality rate (31% per wk) due to causes other than fishing.

All small enclosure estimates were considered to comprise natural mortality plus death due to handling and repeated catching. The rate derived from the culture pond (Expt. 26) included some predation as the pond had a natural lagoon fauna prior to

TABLE II

Mortality estimates from large enclosures and tag-release experiment, based on catch per unit effort.

| Expt. no. | Site | Salinity range (‰) | Temp. range (°C) | Days run | Shrimp category | No. at start | Total no. of recaptures | Mean size | Instantaneous mortality rate | % per wk | Date |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 24 | Caimanero, in | 28–22 | 30–22 | 10 | Uropod clipped | 999 | 139 | 20.6 | 0.004 | 3 | 6 Sept. 1978 |
| 25 | Corral | 28–22 | 30–22 | 10 | Floy tag | 294 | 41 | 23.6 | 0.027 | 17 | 6 Sept. 1978 |
| 26 | Huizache culture pond | 2–4 | 23–25 | 17 | Floy tag | 1382 | 44 | 23.5 | 0.026 | 17 | 13 Oct. 1978 |
| 27 | Huizache after release to lagoon | 4 | 25 | 21 | Floy tag | ca 975 | 324 | – | 0.052 | 31 | 30 Oct. 1978 |

stocking, and potential predators were caught during subsequent sampling (fish and *Callinectes* spp.). All enclosures were vulnerable to predation by birds, but this was considered unlikely in the 5 × 2 m enclosures.

A wide range of mortality rates were recorded from the various experiments, but it is apparent that Floy-tagged shrimp suffered higher mortality in both field and laboratory field tests (Table I and Fig. 2) than untagged individuals. Excluding Experiments 4, 5, 6, and 17, the mean instantaneous mortality rate for tagged shrimp was 0.059, equivalent to a rate of 34% per wk (95% confidence limits: 22–44%). This compares with 0.018 or 12% per wk for untagged shrimp (95% confidence limits: 8–16%).

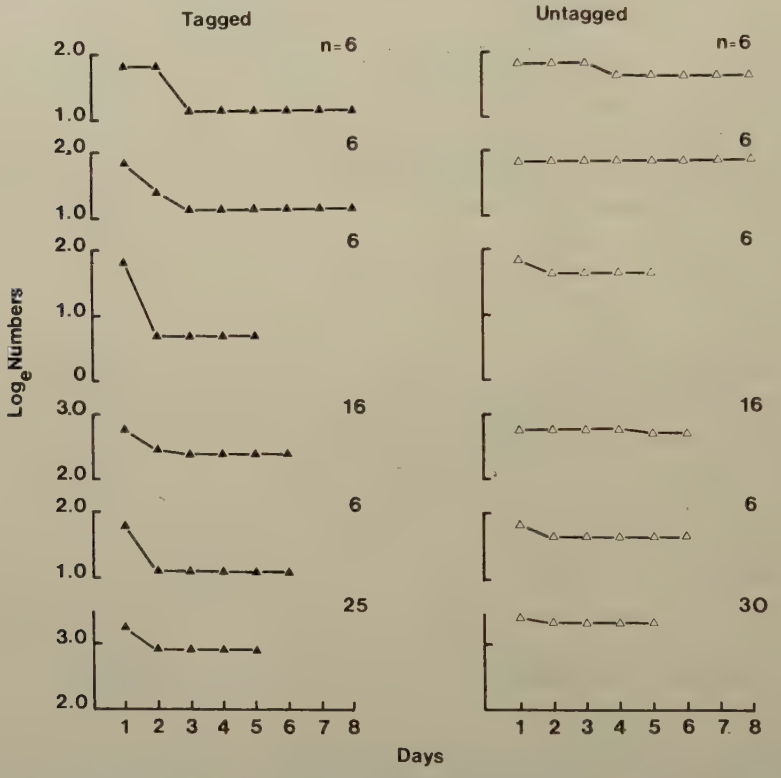


Fig. 2. Mortality of *P. vannamei* under laboratory conditions: n, number of animals.

The very high rates recorded in Experiments 4, 5, 6, and 17 were directly related to changes in environmental conditions. On the night of 24 January 1979, heavy rainstorms in the Sierras behind the lagoon resulted in the flooding of fresh water into the lagoon system during the following week. In the Huizache estero "Ostial", where Experiments 4 and 5 were carried out, this resulted in rapid changes in the salinity (34 to 13‰) and temperature (25 to 10 °C) during the initial stages of the

experiments. Shrimp in Experiment 4 suffered 100% mortality in 6 days, 86% of which occurred within the first 2 days. These shrimp were tagged and released into the enclosure immediately prior to the onset of the rain on 24 January. Experiments 3 and 18, which were already established by this date showed no increase in mortality rate despite the environmental changes. This appears to indicate a high susceptibility to physiological stress on the part of freshly tagged shrimp.

The results of experiment 6 showed a curvilinear relationship between declining numbers and time, with the mortality rate increasing rapidly after 13 days. This also appears to be related to deteriorating environmental conditions. Between Day 15 and Day 22 the water level in the enclosure dropped 10 cm, and the water temperature rose by 6 °C. A dense algal growth encrusted the wire of the enclosure during the last two weeks of the experiment, resulting in reduced circulation and the formation of a surface scum. These same unfavourable conditions are considered to be responsible for the relatively high mortality rate of untagged shrimp in Experiment 17, which was initiated during late February in Caimanero.

Excluding the results from the four experiments discussed above, mortality estimates for untagged shrimp ranged from 3–24% per wk in the field. Laboratory tests (Fig. 2) gave higher rates for both tagged and untagged shrimp, presumably as a result of stress during transport, and subsequent maintenance under aquarium conditions.

The mortality of tagged shrimp in large enclosure experiments was lower than in comparable small enclosure tests (Tables I and II). In the Huizache small enclosures, all shrimp were normally recaptured and measured at least twice each week. This repeated handling probably caused artificially high mortality rates in the tagged shrimp. Untagged shrimp under the same conditions did not, however, suffer such high mortality rates, and it would appear that tagging does increase the susceptibility to handling as well as to adverse environmental conditions.

A comparison of mean shrimp size at tagging with mean initial size of recaptures from the large scale tagging experiment did not indicate any size-dependent factor in mortality over the size range used (Table III).

In order to reduce handling during tagging, sex was not initially determined in the culture pond experiment. The ratio of male to female amongst all the marked shrimp

TABLE III

Mortality in relation to size (carapace length in mm) from large scale tagging experiment.

| | Mean size of original tagged population | Mean original size of recaptures (c.l. in mm) | | | | |
|---|---|---|---|---|---|---|
| | | 22 days | 26 days | 28 days | 31 days | 34 days |
| | 23.54 | 24.3 | 23.3 | 23.3 | 23.2 | 23.7 |
| *s* | 2.10 | 1.85 | 1.65 | 1.78 | 2.04 | 1.80 |
| Number | 1589 | 31 | 25 | 35 | 63 | 15 |

recaptured from the commercial fishery, where sex was recorded, was 160 : 149, and it seems reasonable to assume that mortality was not related to sex. Menz (1976) found a 1 : 1 sex ratio in natural populations of *P. vannamei* from the Caimanero–Huizache system.

## Discussion

Post-larval penaeids enter the lagoon system from the sea via artificially enhanced channels or esteros (Fig. 1). During April to July, these channels are open, but in mid-August barriers called tapos are closed across the esteros to prevent the emigration of juvenile shrimp to the sea. The fishery is thus, effectively, total each year. The tapos are opened only when the numbers of shrimp left in the system are very low. This is normally in early February in Huizache, and April in Caimanero. The tapos are traditonally opened in late March–April.

Total mortality of lagoon shrimp may thus be expected to approach 100% during a fishing season. This was indicated by the assessment of Sepulveda (1976) who calculated a total mortality maximum of 2.39 ($Z$), equivalent to 90% on a monthly basis, in Caimanero lagoon. The natural mortality component may, however, be lower than previously suggested. Edwards (1977) recorded a natural mortality rate of 41% per wk for *P. vannamei* in an enclosure in Caimanero lagoon. The author stated, however, that this may be a high estimate due to escapes. In addition, he used a beam-trawl for exhaustive recapturing on a regular basis, and comparisons made during the present work indicated that initial mortality was higher in beam-trawled than in cast-net caught shrimp (Blake, unpubl.). The rate of 12% per wk obtained from small enclosures in the present study probably reflects a combination of better conditions, less damaging sampling methods, and easier prevention of escapes. It is relevant to emphasize that estimates of mortality made during this work apply only to shrimp in the size range 17–25 mm carapace length (81.5–117.5 mm total length), and may only be applicable to the conditions existing at the time of the experiments, September to March. Edwards (1978) noted that marked changes in environmental conditions occur during the course of a year in the lagoon, as is confirmed by recent hydrological studies (Moore, 1979).

The adverse effects of changing and deteriorating conditions were also noted during the present work in Experiments 6 and 17 (Table I), and this serves to emphasize that natural mortality rates undoubtedly change during the course of a year, and from year to year, according to prevailing conditions. During the 1977–1978 fishing season, accumulations of dead shrimp were noted in front of Tapo Poso de la Hacienda (Fig. 1) as a result of overcrowding. This phenomenon did not occur in the following season.

The effect of predation on natural mortality rates is difficult to assess. In the large scale tag-release experiment, a natural mortality rate of 17% per wk was recorded

whilst the shrimp were held in the culture pond. The maximum natural mortality rate after release to the open lagoon was subsequently calculated as 31% per wk from fishermens' returns. This is, however, based on the assumption that the fishermen both captured and returned all the tagged shrimp remaining alive in the lagoon. While co-operation was extremely good, and 33% of tagged shrimp released to the lagoon were ultimately returned, some tags were probably lost. Similarly, poaching is a serious problem on the Huizache lagoon, and may have resulted in loss of tagged shrimp. No tag shedding was observed either in aquaria or small enclosures. The rate obtained in the culture pond was in the presence of potential predators, and it is unlikely that release to the open lagoon would have increased natural mortality from 17–31% simply by predation pressure.

From the small enclosure experiments it was apparent that tagged shrimp are more vulnerable to death from natural causes and experimental handling than untagged shrimp. In the open lagoon, this vulnerability may be emphasized as a result of selective predation. Analysis of the stomach contents of some species of aquatic birds, notably cormorants, revealed the presence of penaeids (Almada, in prep.). The presence of a bright orange marker would presumably increase the chances of detection by avian predators. Thus, while the mean mortality rate for untagged shrimp in small enclosures (12% per wk) may be a low estimate owing to the absence of predators, the maximum rate of 31%, calculated for the open lagoon, is artificially high. The actual natural mortality rate for untagged and unhandled shrimp in the open lagoon, of the size range specified, during the period September 1978 to March 1979, must lie between these two estimates, with a strong bias towards the lower end of the range. The 17% per wk value for shrimp in the culture pond, and untagged shrimp in the large enclosure, may represent the most realistic estimate, particularly as both types of experiment were susceptible to predation, at least by birds.

Studies of predation by fish in the lagoon system have been made by K. Warburton (pers. comm.) and are in progress (G. Diaz, pers. comm.). Several species have been found to take penaeids, notably *Galeichthys caerulscens*, *Centropomus robalito*, *Elops affinis* and *Polynemus* sp. These fish are, however, concentrated principally in the deeper estero regions, and are unlikely to contribute significantly to natural mortality in the open lagoon. They do, however, take large numbers of shrimp when these are concentrated in front of the tapos during emigration runs, and may thus influence the total capture of shrimp in these places at such times. It is more likely that the avian predators exert an influence on natural mortality over the lagoon as a whole, for a longer proportion of the overall recruitment and fishing period.

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# EFFECTS OF SIMULATED UPWELLING AND OLIGOTROPHY ON CHEMOSTAT-GROWN NATURAL MARINE PHYTOPLANKTON ASSEMBLAGES[1]

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**Abstract:** Natural phytoplankton assemblages from the Scripps Pier were grown in two chemostats under conditions that simulated two rates of upwelling followed by oligotrophic conditions. At a moderate upwelling rate ($D = 0.3 \cdot \text{day}^{-1}$) centric diatoms were selected, while at a low rate ($D = 0.1 \cdot \text{day}^{-1}$) a mixture of species dominated. Pumping of low-nutrient water (oligotrophy) resulted in a mixture of species at both rates. Upwelling at a high rate decreased diversity of the crop as compared with the low rate or oligotrophy. These results are compared with those of others who have subjected natural assemblages to continuous culture.

## INTRODUCTION

Upwelling of nutrient-rich, cool water is a common phenomenon in coastal waters and has received a great deal of study in recent years. Upwelling leads to increases in phytoplankton productivity and standing crop due to nutrient input and is one of the factors that in turn leads to productive fisheries in coastal waters. The process is "quasi" (Walsh, 1975) and varies with the wind stress along the coasts. When upwelling ceases or the water moves offshore and becomes diluted with low-nutrient sea water oligotrophic conditions prevail.

In a previous paper, Dodson & Thomas (1977) described the growth and survival of two marine phytoplankton cultures during simulated upwelling followed by oligotrophy. In these experiments the algae were held in dialysis bags of 7-$\mu$m mesh Nitex netting and high- and low-nutrient water was pumped past the cultures over a period of 80 days. The algae grew well during simulated upwelling and survived a long period of low-nutrient conditions.

Dodson & Thomas (unpubl.) have used smaller-mesh membranes (1 $\mu$m pore size) in dialysis bags to retain nannoplankton in order to determine if selection for various species in a mixed algal population exists. In long-term studies (up to $2\frac{1}{2}$ months), attempts to use small pore-size membranes were plagued with difficulties.

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Nuclepore membranes were fragile and developed holes, while Gelman Acropore membranes became clogged by bacterial growth. Dye diffusion tests showed that, when the membranes were clogged, equilibrium on both sides of the membrane was not achieved in a period as long as 2 days. In a comparison of these dialysis bags with a chemostat arrangement, crop changes in the chemostat were rapid under these simulated upwelling and oligotrophic conditions and there was really no need to enclose the cultures in a dialysis bag. In a chemostat, as opposed to dialysis culture, washout of cells occurs and this corresponds to natural losses such as grazing and sinking of cells. However, it should be recognized that washout is a uniform process, while in nature grazing and sinking are non-random events.

Previously Dodson & Thomas (1977) described conditions off Baja California in 1964–1966 where diatoms and/or dinoflagellates persisted in upwelled water, while the latter were found in offshore oligotrophic water. Blasco (1977) described a bloom of red-tide dinoflagellates in the same upwelling area in 1972. In 1977, at the Scripps Pier, upwelling in March resulted in a mixed bloom of diatoms and dinoflagellates (Thomas & Seibert, unpubl.). Blooms of dinoflagellates alone were not associated with upwelling in 1977 Pier samples. In reviewing historical phytoplankton data (1928–1939) from the Pier, Tont (1976) noted that diatom blooms were associated with upwelling.

The purpose of this paper is to examine the hypothesis that diatoms will dominate over other algae when upwelling conditions prevail, and that other algae may predominate under oligotrophic conditions. A natural assemblage from the Scripps Pier was brought into the laboratory and subjected to two different rates of upwelling which were followed by low-nutrient, oligotrophic conditions. Because of the difficulties with dialysis cultures mentioned above, the natural assemblage was grown in two chemostats.

## Materials and Methods

On 31 October, 1977, a water sample containing natural phytoplankton was collected from the Scripps Pier at high tide to avoid contamination with sand from the surf zone. The dominant algae in the sample were small ($\approx 5$ $\mu$m diameter) green flagellates, but other species of centric and pennate diatoms and dinoflagellates were present. The sample was filtered through 200-$\mu$m mesh Nitex netting to remove large zooplankton. This filtration probably did not remove small zooplankton.

Three liters of sample were placed in each of two chemostats, A and B. These consisted of spherical glass reaction vessels which had water jackets. There were ports for inoculation, sampling, overflow, aeration, and medium inflow. The chemostats were stirred slowly by magnetic stirring bars. Light was supplied by a bank of cool white fluorescent tubes at an intensity of 100–400 $\mu$Einstein $\cdot$ m $^{2} \cdot$ sec $^{-1}$ of photo-

synthetically active quanta. The photoperiod was 12 L : 12 D and the temperature was 20 °C. The temperature of the water at the Scripps Pier was 18 °C.

The samples were equilibrated without pumping for 24 h. The pumping of enriched sea-water medium was commenced at 1100 ml · day $^{-1}$ ($D = 0.3 \cdot \text{day}^{-1}$) in Chemostat A and 300 ml · day $^{-1}$ ($D = 0.1 \cdot \text{day}^{-1}$) in Chemostat B. The rate in A corresponds to an upwelling rate of 10 m per day into a 30-m euphotic zone found off Baja California by Walsh *et al.* (1974), while the rate in B represents weak upwelling.

The enriched sea-water medium contained 29.4 $\mu$M soluble Si, 2.10 $\mu$M $PO_4$-P, 1.70 $\mu$M $NH_4$-N, and 33.8 $\mu$M $NO_3$-N. These major nutrient concentrations were similar to those found in Baja California upwelled water (Walsh *et al.*, 1974). Vitamins, trace metals and Fe sequestrene were added at one-tenth the concentrations of Guillard's "f" medium (Guillard & Ryther, 1962).

After pumping enriched sea water for 8 days, the medium in A and B was changed to low-nutrient sea water pumped in at the same rate to simulate a slow change to oligotrophic conditions. This sea water contained 13.0 $\mu$M soluble Si, 0.52 $\mu$M $PO_4$-P, 0.68 $\mu$M $NH_4$-N, and 0.7 $\mu$M $NO_3$-N and had been collected from the sea surface well offshore. The experiment was terminated after 1 month.

A daily sample of 250 ml was taken from each chemostat. One hundred ml were frozen for nutrient analyses, and an additional 100 ml were preserved with Lugol's $I_2$ solution for phytoplankton identification and enumeration. In vivo chlorophyll fluorescence (Lorenzen, 1966) was measured in the remaining sample to follow growth in the chemostats.

Nutrient analyses were carried out by automated methods (Head, 1971; Strickland & Parsons, 1972). Phytoplankton were settled overnight for identification and enumeration, using an inverted microscope (Utermöhl, 1958). Phytoplankton carbon in each species was computed from the numbers of cells and the cell volume as previously described (see Thomas & Seibert, 1977, for details). The species were then grouped into centric diatoms, pennate diatoms, dinoflagellates, and unidentified microflagellates, and the carbon in each group was calculated.

Media were filtered sterilized and sterile techniques were used in sampling, but the cultures were not bacteria-free, since a natural water sample was used. There was no appreciable growth of algae on the walls of the vessels during the course of the experiment, but there was undoubtedly bacterial growth on the walls.

## Results

Nutrient concentration changes are shown in Fig. 1. During the first few days of pumping of enriched sea water through the chemostats, nitrate, phosphate, and soluble silica increased greatly. Ammonium increased somewhat later. The changes were generally greater in the chemostat pumped at the high rate (A) than in the chemostat pumped at a slower rate (B). After 5 November, 1977, the nutrients

(especially nitrate) had decreased greatly, as the crop took them up and grew. Pumping with low-nutrient sea water resulted in continued low concentrations except for soluble Si. The latter may have been regenerated as the crop decreased. Regeneration of N as ammonium possibly occurred on 7 November when an increase in this nutrient was found.

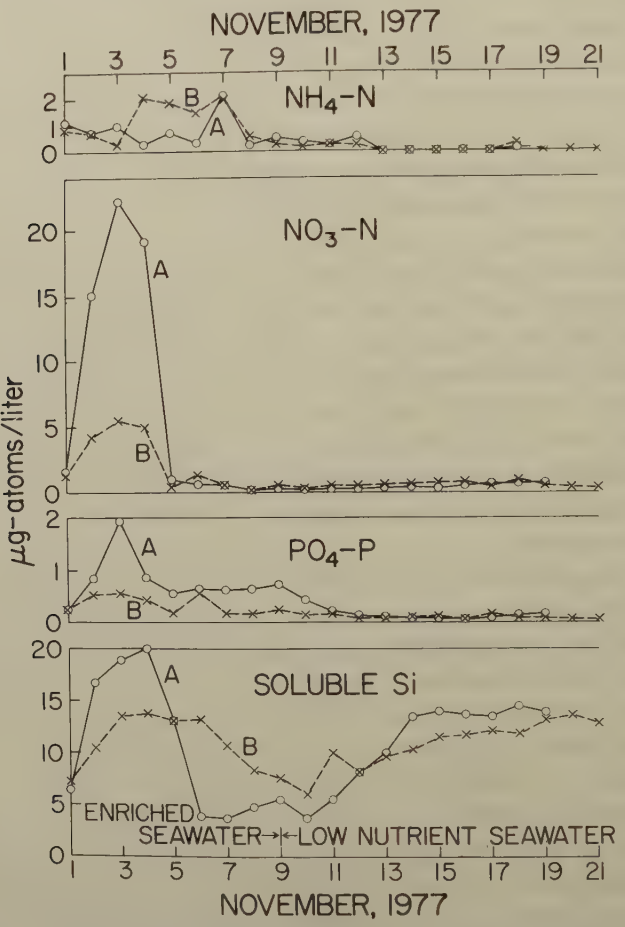


Fig. 1. Changes in nutrients during simulated upwelling followed by oligotrophy: A, high pumping rate ($D = 0.3 \cdot \text{day}^{-1}$); B, low pumping rate ($D = 0.1 \cdot \text{day}^{-1}$).

As nutrients were taken up between 3 November and 5 November, the crop increased greatly (Figs. 2 and 3) and reached a steady state. As might be expected, the maximum crop in A was over twice that in B. There was a sharp decrease in crop carbon (Fig. 3) on 8 November in both chemostats. This decrease was not found for in vivo fluorescence.

Following the onset of pumping of nutrient-poor water, fluorescence values (Fig. 2) remained in the steady state for a few days (especially in B) and then decreased.

Carbon (Fig. 3) fluctuated in B before the decrease commenced. The decrease in fluorescence may be due to a metabolic adjustment to nutrient-poor conditions, but eventually the decrease was due to a cessation of growth and washout of the cells as indicated by the results for phytoplankton carbon. The crop decreased at a slower rate in B than in A. This was expected, since the dilution rate in B was less than that in A.

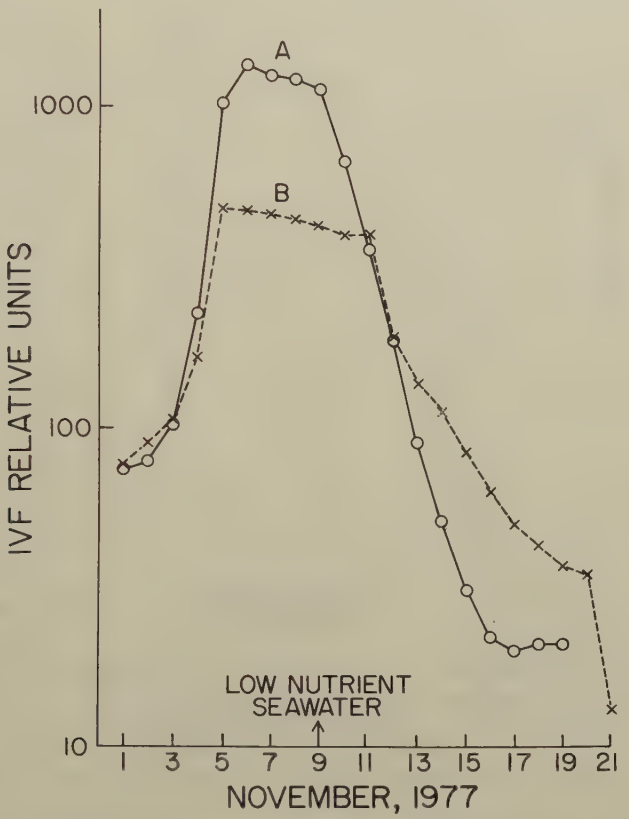


Fig. 2. Changes in in vivo fluorescence: A, $D = 0.3 \cdot \text{day}^{-1}$; B, $D = 0.1 \cdot \text{day}^{-1}$.

Ratios (Table I) of phytoplankton carbon to relative in vivo fluorescence (a rough measure of the carbon/chlorophyll ratios) increased as the crops increased and were at a maximum at steady state 2 days following the onset of oligotrophic conditions. These ratios then decreased but not as much in B as in A. High ratios may be indicative of N deficiency at steady state, but the ratios decreased with oligotrophic conditions, and thus this explanation of the variation in the ratios may or may not be correct.

One of the goals of this experiment was to see which phytoplankton were selected by upwelling conditions and which by oligotrophic conditions. Fig. 4 shows the carbon in each of four algal groups in Chemostat A. During exponential growth, unidentified microflagellates increased until 5 November. However, there was a strong

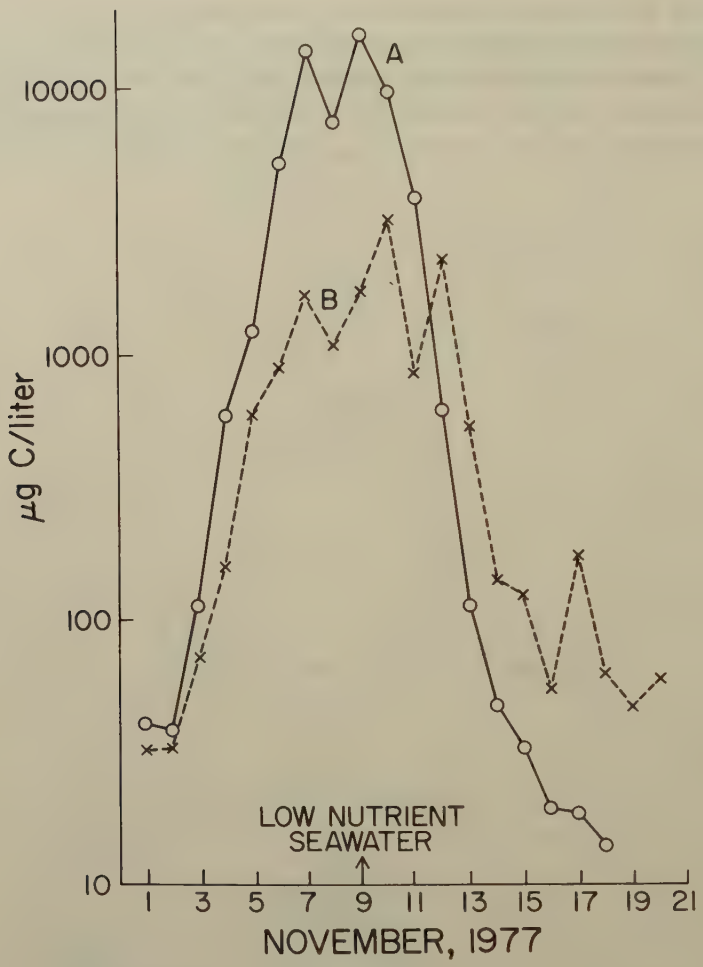


Fig. 3. Changes in phytoplankton carbon: A, $D = 0.3 \cdot \text{day}^{-1}$; B, $D = 0.1 \cdot \text{day}^{-1}$.

selection for centric diatoms under upwelling conditions, and these algae persisted in the steady state. During this period these algae made up more than 85% of the phytoplankton carbon. Initially only 2% of the crop consisted of this group. The steady state crop consisted mainly of the diatoms *Chaetoceros decipiens*, other *Chaetoceros* spp., and *Thalassiosira* spp. During pumping of oligotrophic sea water, dominance was shared by centric diatoms and microflagellates. Other algae rarely made up more than 10% of the algal carbon during the whole experiment, although pennate diatom carbon increased somewhat under eutrophic conditions.

Selection of algae differed in Chemostat B (Fig. 5) from that in A. Microflagellates were dominant during the initial stages of exponential growth. Both centric and pennate diatom carbon also increased greatly. At steady state, dominance was shared

TABLE I

Ratios of phytoplankton carbon to relative in vivo fluorescence in chemostat-grown natural assemblages: IVF, in vivo fluorescence; C/IVF, phytoplankton carbon/in vivo fluorescence ratio.

| Date | Chemostat A | | | Chemostat B | | |
|---|---|---|---|---|---|---|
| | $\mu g\ C \cdot l^{-1}$ | IVF | C/IVF | $\mu g\ C \cdot l^{-1}$ | IVF | C/IVF |
| Oct. 31 | 39.9 | 74 | 0.54 | 39.9 | 74 | 0.54 |
| Nov. 1 | 40.5 | 74 | 0.55 | 32.3 | 75 | 0.43 |
| 2 | 37.4 | 79 | 0.47 | 32.3 | 90 | 0.36 |
| 3 | 110.7 | 101 | 1.10 | 71.7 | 109 | 0.66 |
| 4 | 584.8 | 226 | 2.59 | 159.0 | 165 | 0.96 |
| 5 | 1201.4 | 1012 | 1.19 | '600.0 | 481 | 1.25 |
| 6 | 5156.3 | 1378 | 3.74 | 882.0 | 475 | 1.86 |
| 7 | 13274.5 | 1233 | 10.77 | 1680.2 | 464 | 3.62 |
| 8 | 7215.5 | 1201 | 6.01 | 749.5 | 443 | 2.14 |
| 9 | 15168.3 | 1107 | 13.70 | 1703.6 | 432 | 3.94 |
| 10 | 9367.2 | 669 | 14.00 | 3180.5 | 399 | 7.97 |
| 11 | 3726.7 | 356 | 10.47 | 842.7 | 399 | 2.11 |
| 12 | 604.1 | 185 | 3.27 | 2205.3 | 191 | 11.55 |
| 13 | 111.8 | 90 | 1.24 | 535.8 | 137 | 3.91 |
| 14 | 47.4 | 51 | 0.93 | 139.2 | 112 | 1.24 |
| 15 | 32.2 | 31 | 1.04 | 122.6 | 84 | 1.46 |
| 16 | 19.5 | 24 | 0.81 | 55.3 | 64 | 0.86 |
| 17 | 18.5 | 20 | 0.93 | 175.4 | 50 | 3.51 |
| 18 | 14.0 | 22 | 0.74 | 63.7 | 43 | 1.48 |
| 19 | 7.0 | 21 | 0.33 | 46.8 | 37 | 1.26 |
| 20 | – | – | – | 60.8 | 35 | 1.74 |

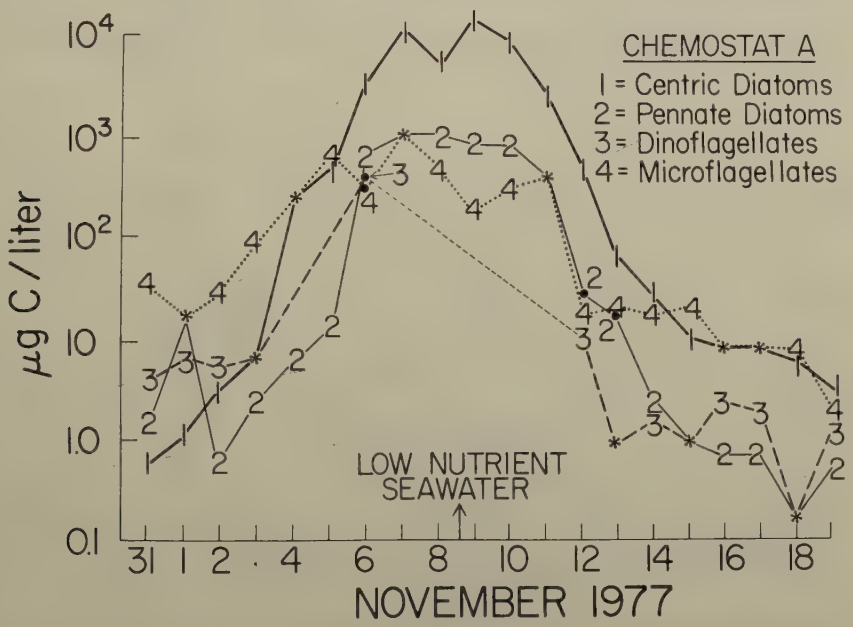


Fig. 4. Changes in major phytoplankton groups, Chemostat A: $D = 0.1 \cdot day^{-1}$.

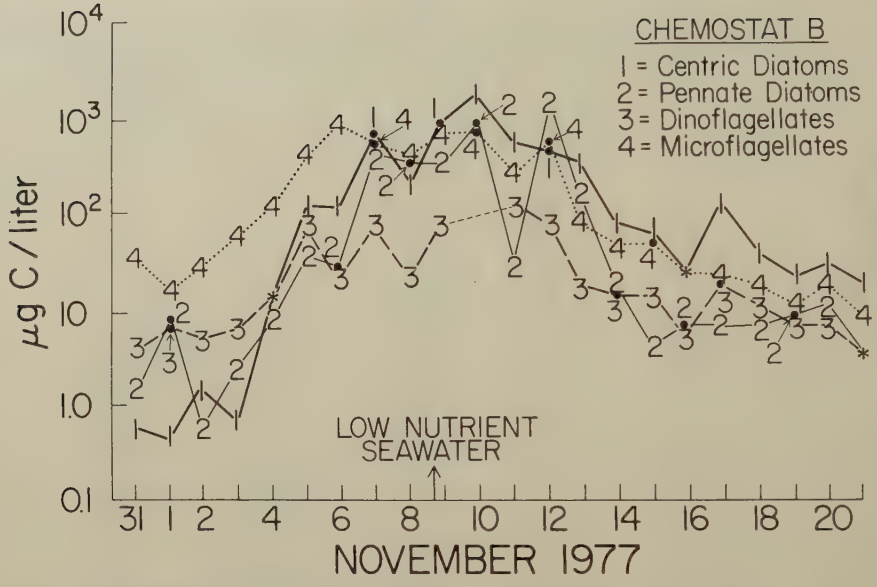


Fig. 5. Changes in major phytoplankton groups, Chemostat B: $D = 0.1 \cdot \text{day}^{-1}$.

among these three groups, and the algae that were dominant consisted mainly of *Chaetoceros* spp., *Nitzschia* spp., and small (2–4 μm) flagellates. Under oligotrophic conditions pennate diatoms were abundant at first but decreased greatly, so that by 15 November they made up only 2% the crop. Late in the experiment centric diatoms were dominant, but only slightly. Dinoflagellates never became an important portion of the crop carbon in either chemostat.

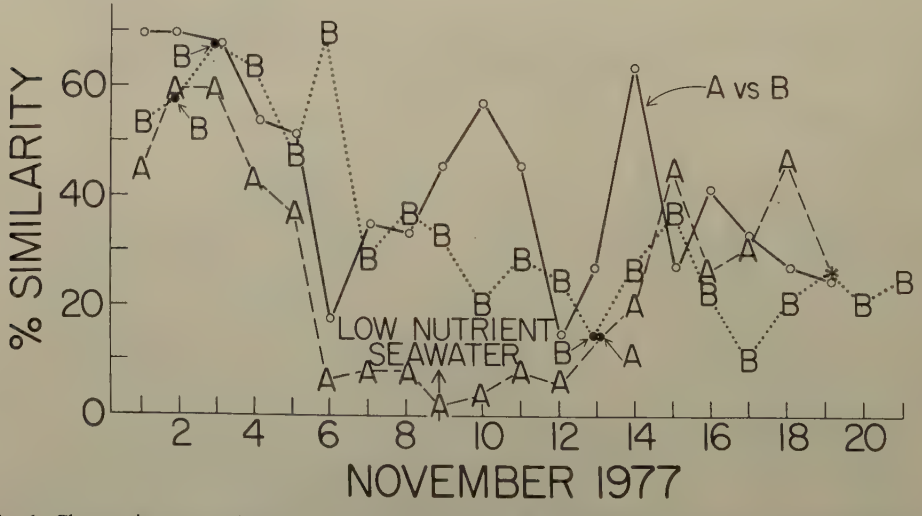


Fig. 6. Changes in percent similarity: A, percent similarity in Chemostat A compared to the initial crop; B, percent similarity in Chemostat B compared to the initial crop; ○, percent similarity of A versus B.

Further documentation of these changes is given in Fig. 6, which consists of plots of the degree of similarity expressed as a percentage (Whittaker, 1960) between the initial sample and the community in Chemostats A and B and between A and B. Initial containment without pumping for a day resulted in a change in the communities in both A and B, so that the percent similarity was about 50% on 1 November. One day of pumping resulted in some increase in the percentage. Thereafter, as centric diatoms bloomed in A, the similarity to the initial crop decreased very rapidly and was only 2–6% during steady state. The assemblage became more similar to the initial community during oligotrophic pumping. While the percent similarity decreased in B as compared to the initial crop, the change was not as great as in A, since dominance was shared by centric diatoms, pennate diatoms, and microflagellates in B. The assemblages in A and B were very dissimilar at steady state and then became more similar during early oligotrophy with a peak in the index (A vs. B) on 14 November.

In Chemostat A, crop diversity (Shannon & Weaver, 1963) remained high during initial simulated upwelling, but decreased until steady state was reached and the diversity index was at a minimum (Fig. 7). The number of taxa was 25 initially and

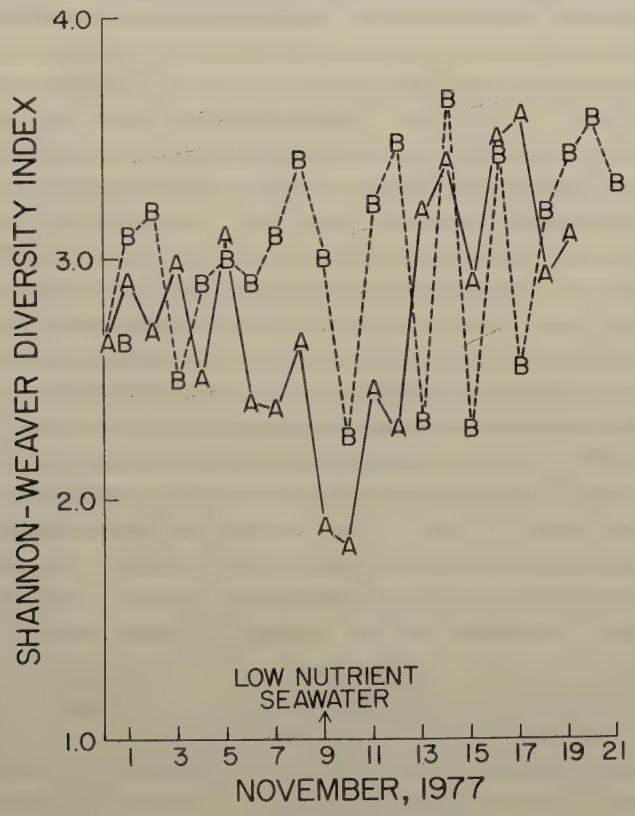


Fig. 7. Shannon–Weaver diversity indices in Chemostats A and B.

decreased to 11 on 10 November. Thereafter, with oligotrophic pumping, the index and the number of taxa increased. The diversity of the assemblage in Chemostat B was generally greater even at steady state than in A, and there were sharp day-to-day changes in the index in B under oligotrophic conditions.

## Discussion

The most important result of this experiment was the strong selection of centric diatoms during moderate ($D = 0.3 \cdot \text{day}^{-1}$) upwelling, as contrasted with selection for some centric diatoms but also pennate diatoms and microflagellates during low ($D = 0.1 \cdot \text{day}^{-1}$) upwelling. Oligotrophic conditions resulted in a mixture of species in both chemostats. Selection of species during moderate upwelling resulted in a decrease in diversity, as contrasted with a greater diversity under low upwelling conditions or oligotrophic conditions.

These observations fit in well with the concept of Turpin & Harrison (1979) relating species composition of natural assemblages to specific nutrient flux. They noted that at a high flux centric diatoms would dominate; and at a low flux, flagellates would dominate. This concept was verified also by Harrison & Davis (1979), who showed that the centric diatoms, *Skeletonema* and *Chaetoceros*, dominated at a high dilution rate in an outdoor N-limited chemostat, while at a low dilution rate the percent of flagellates increased and there was a codominance with larger slower-growing diatoms. In continuous cultures of natural populations, Mickelson (in press) noted that small diatoms dominated at all of five dilution rates, but that certain species (*Skeletonema*, *Chaetoceros septentrionale*, and *Nitzschia delicatissima*) dominated at high rates, while others (*N. delicatissima*, *Leptocylindrus*, *Cylindrotheca*, and *Chaetoceros sociale*) dominated at lower rates. Moderate nutrient enrichment of a large plastic enclosure controlled experimental ecosystem, moored at the CEPEX site in Saanich Inlet, British Columbia, resulted in a maintenance of a *Chaetoceros* bloom, while no enrichment resulted in flagellate dominance (Parsons *et al.*, 1977). In another CEPEX experiment with large plastic enclosures, it was noted that *Chaetoceros* and *Thalassiosira* decreased when N levels became lower than 1 $\mu$g-at. $\cdot l^{-1}$, but that healthy *Leptocylindrus* persisted for approximately 1 wk under N-limitation and could form spores when N was added (Davis *et al.*, in press).

The above results were based on N-limitation. Dunstan & Menzel (1971) noted that enrichment of continuous natural cultures with sewage effluent or a complete medium resulted in no particular species selection, but maintained species that were already present, in this case centric diatoms. Similar results were obtained by Jones *et al.* (1978) under conditions of P-limitation, that is, the species composition did not vary greatly in continuous P-limited cultures of natural phytoplankton (mainly centric diatoms) from that found in the sea loch from which the assemblage was taken.

In our experiment, nutrient enrichment (pumping) was continuous, while upwelling in nature may be intermittent depending on the wind forces. Discontinuous enrichment has been shown to select for species that are different from those selected under continuous enrichment (Turpin & Harrison, 1979). These authors showed that continuous enrichment in an N-limited chemostat (natural assemblage) selected *Chaetoceros*, while enrichment with $NH_4^+$ only once a day selected *Skeletonema*. With intermediate patchiness both diatoms codominated. We might also have observed different selection if enrichment had been sporadic.

Allelopathic interactions among algal species may be superimposed on selection by nutrient flux changes. Thus, Thomas & Hastings (unpubl.) found that *Skeletonema* would inhibit the growth of a small green flagellate in mixed culture. Furthermore, filtrates of *Skeletonema* would inhibit the growth of the flagellate. Recently it was shown that *Chaetoceros decipiens* will inhibit the growth of *Carteria pallida*, a green flagellate, in mixed culture (Thomas & Alden, unpubl.). Both organisms were isolated from Scripps Pier samples.

These results may have been biased by an unknown grazing factor since microzooplankton were probably not excluded by the initial filtration through 200-$\mu$m netting. However, few microzooplankton, such as ciliates, tintinnids, etc., were ever observed during the microscopic identification and enumeration of the phytoplankton. Thus, we believe that the changes in the assemblages were entirely due to nutrient changes.

The present results do not explain dinoflagellate dominance in upwelling areas (Blasco, 1977). Possibly if we had carried out our experiment during that portion of the year – spring or summer – when the initial inoculum had contained a dominant dinoflagellate population, we would have selected for this group of species. On the other hand, conditions in our chemostats – aeration plus stirring – may have been too turbulent for maximum dinoflagellate growth.

This experiment deserves repeating with other seed populations to see if species composition of the initial population is the crucial factor in selection of dominant species. Also the experiment should be continued through several simulated upwelling cycles to see whether these results represent general phenomena that might have predictive value in explaining the dominance of various species during natural upwelling cycles in the sea.

## Acknowledgements

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# CANONICAL CORRELATION ANALYSIS OF MARINE MACROBENTHOS SURVEY DATA

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**Abstract:** Canonical correlation analysis is applied to measurements of environmental variables and species distributions made during a survey of macrobenthos around a sewage-treatment farm drain. The implications of data reduction, necessary to enable the method to proceed, are discussed. The amount of data was reduced by discarding the rarest species, discarding species occurring at fewest stations, and including only those species and environmental variables which correlated highly with the greatest number of other variables. Only the third data-reduction scheme gave ecologically sensible results. Use of station scores on the first two canonical variates (CV1 and CV2) enabled the sampling grid to be divided into a group of nearshore stations, a group of intermediate depth, and a group of deep offshore stations. Loadings of environmental variables on the canonical variates were found to be unstable but correlations between these variables and canonical variates enabled the variates to be interpreted: CV1 as a gradient of depth and associated changes in sediment characteristics, CV2 with depth- and nutrient-related components, and CV3 as patchiness in sediment characteristics different from that normally expected with depth. Use of correlations between species and canonical variates enables definition of two major species groups, one confined to nearshore environments and a second offshore. These groups (and their sub-groups) related well to groups defined previously by hierarchical classification. It is concluded that, with careful attention to the method of data reduction, canonical correlation analysis can be an effective tool in the analysis of marine benthic survey data.

## INTRODUCTION

Descriptive ecological surveys are often undertaken with the stated objective of describing the distribution of plants and animals in terms of physico-chemical features of the environment. To this end, surveys are designed to collect information divided a priori into two sets, biotic data and abiotic data. But in spite of the stated objective and possession of data divided conveniently into two parts, ecologists have not been particularly successful in relating biotic and abiotic data. In marine benthic ecology where complex statistical methods are widely used there has been little progress in this field. Although benthic surveys have frequently been carried out as part of marine environmental research, relationships between animal distributions and environmental disturbance or pollutants have been rarely shown other than by subjective methods.

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Mathematical methods for looking for pattern in sets of data of a single type are well developed. Classification and ordination have been widely applied with success to the biological half of the data, usually counts of species in samples. Data on abiotic variables have been treated separately and differently or not at all. The basic problem of reducing the two data sets to a comprehensible size and then relating them in a meaningful way has rarely been considered in marine benthic ecology.

Canonical correlation analysis is a statistical method conceptually appealing in its ability to ascertain the extent to which one set of measurements is related to another and to determine the particular attributes which have been responsible for these relationships. The method, developed by Hotelling (1935), is an extension of principal components analysis.

Linear functions of the two sets of variables are selected so that the (canonical) correlation between the two functions is maximized. Furthermore, pairs of linear functions that are uncorrelated with previous pairs and have the maximum correlation possible may also be found. Geometrically, the method looks at the relative positioning of the subjects in the two measurement spaces (Cooley & Lohnes, 1971, p. 169). Usually the variables with the highest coefficients in each of these linear functions are assumed to define that function and hence the key features relating the two data sets may be assessed from a pair of coefficient vectors (Pielou, 1969; Cooley & Lohnes, 1971; Clifford & Stephenson, 1975; Williams, 1976).

Canonical correlation analysis has not been especially useful in agriculture or in biology in general (Austin, 1968; Williams, 1976) although Barkham & Norris (1970) and McIntire (1978) found its use encouraging. The method suffers, like many other similar parametric techniques, from the assumption of linear relationships between the variables. Its successful use in this paper may be due to the fact that sampling was from a fixed point on shore (i.e. the drain) seawards.

Cassie & Michael (1968) used canonical correlation analysis on data from a marine intertidal mudflat. Their results took the form of counts of 8 species and 9 sediment parameters for 21 stations. It is more usual in benthic surveys to find many more species than there are stations, in which case the corresponding correlation matrix will be singular and hence no solution will be available. The canonical correlations will be unity for the first one or more pairs of canonical variates calculated. This means that the canonical correlations can be made to appear high simply by making the number of variables included approach the number of stations.

It is therefore necessary to reduce the number of species (and possibly also the number of environmental variables); this may be done in several ways. The simplest approach is to discard the rarest species or species which occur at fewest stations. Species whose distributions correlate weakly with those of other species are unlikely to contribute to an overall pattern and may, therefore, be ignored (Williams, 1976). Species which do not correlate highly with any environmental variable, or vice versa, may be omitted.

This paper describes the use of canonical correlation analysis of data from a marine benthic survey which has previously been analysed using classification and partial correlation (Poore & Kudenov, 1978). The objective then was to describe the distribution of the fauna in terms of abiotic variables and in particular to assess the effect of a sewage-treatment farm drain on the fauna. In this paper this is still an objective, but the main one is to illustrate the stages involved in applying canonical correlation analysis.

## METHODS

### SAMPLING

A 2-km$^2$ area was sampled at 36 stations on seven radiating lines during 18–28 November 1975 (Fig. 1). The origin of the grid was near the 145W drain of the Wer-

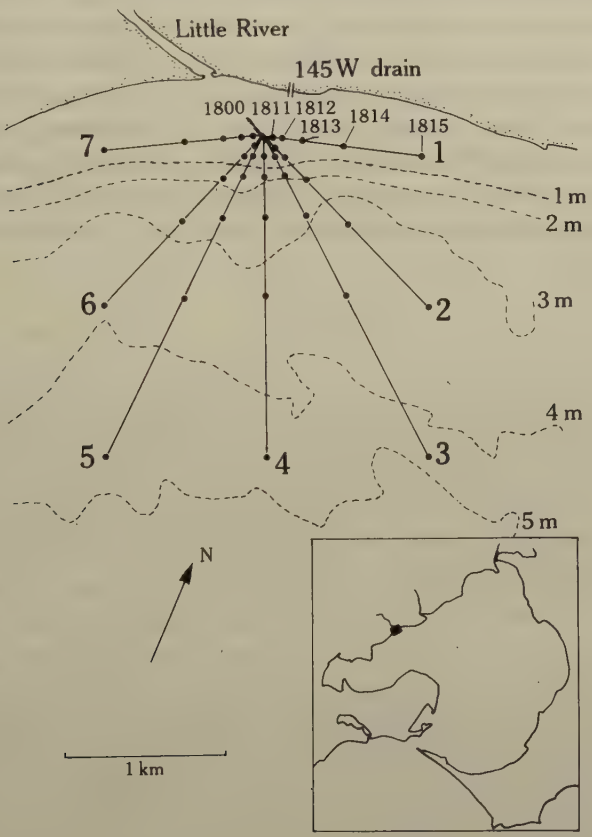


Fig. 1. The area studied and its location in Port Phillip Bay (inset): transects are numbered 1 to 7; the origin (Station 1800) and stations on transect 1 (1811 -1815) are labelled (Poore & Kudenov, 1978); the station prefix 18 is not used in subsequent figures.

ribee sewage-treatment farm. About 40 environmental variables were determined and macrofaunal species in 0.05-$m^2$ samples were counted (Poore & Kudenov, 1978).

## DATA REDUCTION

Thirteen abiotic variables were selected: depth, concentrations of nitrite plus nitrate; ammonia and phosphate in the interstitial water; percentage of carbonate in the sediment; mean particle size; sorting coefficient; percentages of medium sand, fine sand, very fine sand, silt and clay; and percentage of organic matter (Poore & Kudenov, 1978). The list of species was reduced from 246 to 49 first by omitting all species averaging less than one individual per sample.

Three alternative schemes were tried to further reduce the number of variables and canonical correlation analysis was performed on each resulting data set. (1) Species with total counts of fewer than 118 individuals were discarded; this left 22 species and all 13 environmental variables. (2) Only species present in half or more of the stations were included; this resulted in 18 species and 13 environmental variables. (3) Only species which correlated with three or more environmental variables with $|r| > 0.4$ were included. In addition, only environmental variables correlating with seven or more species with $|r| > 0.4$ were included. This process left 22 species and 9 environmental variables in the analysis. It was observed that the species which were omitted were those which also correlated highly with fewest ($< 14$) other species and the environmental variables omitted correlated highly with at most two other environmental variables.

## DATA TRANSFORMATION

As with most multivariate methods, more success can be expected when applying canonical correlation analysis if the data are approximately multivariate normal in distribution. As counts are usually skewed in benthic surveys, a log transformation was decided upon. In fact, log (count + 1) was used. In the case of abiotic variables, those that were percentages ($P$) had the transformation arctan square root applied to $P/(100 - P)$ (Snedecor & Cochran, 1976, p. 32).

## DATA ANALYSIS

For each reduced set of $p$ species variables and reduced set of $q$ environmental variables a canonical correlation analysis was carried out using the SPSS subprogram CANCORR. The output consisted of (1) correlation coefficients between all pairs of $p + q$ variables; (2) correlations between $q$ pairs of linear functions (canonical variates); (3) coefficients (loadings) of the $p + q$ original variables on the $q$ pairs of canonical variates (CVs), and (4) the canonical variate scores for the stations on the first two pairs of CVs.

Considering the first pair of canonical variates for example, if $s_{1i}, s_{2i} \ldots s_{pi}$ are the standardized species counts at Station $i$ and $e_{1i}, e_{2i} \ldots e_{qi}$ are the standardized values of environmental variables at that station, then the output gives the vector of loadings $a$ and $b$ such that

$$z_{1i} = a_1 s_{1i} + a_2 s_{2i} + \ldots + a_p s_{pi}$$

$$z_{2i} = b_1 e_{1i} + b_2 e_{2i} + \ldots + b_q e_{qi},$$

where $z_1$ and $z_2$ are the pair of canonical variates. Hence the canonical variate scores for Station $i$ can be evaluated, plus the correlation between $z_1$ and $z_2$ (called the canonical correlation coefficient).

Given a pair of canonical variates that are highly correlated it is only necessary to consider the canonical variate scores for one set of data variables. Selecting one set of station scores from each of the first two pairs of canonical variates (environmental CVs in our case), the stations were plotted in the two dimensional space, in order to see if they grouped together. These groups were then marked off on the original map of the sampling area in which the stations occurred (see Fig. 1), to see what interpretation, if any, could be placed on the canonical variates.

Given the correlation matrix for the environmental variables (or species), multiplication by the corresponding vector of loadings, produces a vector of correlations between the environmental variables (or species) and the canonical variate. If one or two of these correlations are high, then the canonical variate may be interpreted.

## Results

The initial intuitive interpretation of the results, both environmental and biotic, suggested that the major gradient in the sample grid was with increasing distance from shore and increasing depth. Sediments nearshore (within the 1-m contour) were more sandy, better sorted (lower sorting coefficient) and contained less carbonate than those offshore (Poore & Kudenov, 1978). Interstitial nutrient concentrations were generally greater nearshore than offshore. The stations were also separable into a nearshore group and an offshore group based on their fauna but many species were quite widespread (Poore & Kudenov, 1978).

In the first data reduction scheme both CV1 (the first pair of canonical variates) and CV2 represented several environmental variables but only CV2 was associated with any species. The correlations between these species and environmental variables were not high and were often contradictory in sign. The sample scores plotted for a canonical variate of each pair did not fall into discrete groups or show any spatial trend. The only exception was that the shallowest stations tended to fall together.

Similar results were obtained with the second data reduction scheme although different environmental variables and species were involved. In neither scheme

TABLE I

Loadings on nine environmental variables and 22 species on the first two canonical variates (CV1 and CV2) in the third canonical correlation analysis: species are given with a single-letter code indicating taxon (thus Amphipoda, Bivalvia, Cumacea, Isopoda, Mysidacea, Ostracoda, Polychaeta) and the total number of individuals captured in 36 samples.

| Environmental and biotic variables | | | CV1 $r = 0.993$ | CV2 $r = 0.979$ |
|---|---|---|---|---|
| Depth | | | 0.588 | 0.509 |
| Orthophosphate | | | 0.090 | 0.530 |
| Ammonia | | | 0.119 | −0.316 |
| Percentage carbonate | | | 0.037 | −0.509 |
| Sorting coefficient | | | −0.251 | −0.257 |
| Percentage medium sand | | | −0.354 | −0.585 |
| Percentage fine sand | | | −0.851 | −0.412 |
| Percentage silt | | | −0.330 | −0.199 |
| Percentage clay | | | 0.530 | 0.138 |
| *Gammaropsis* sp. 1 | A | 1414 | −0.072 | 0.021 |
| *Mediomastus* sp. 1 | P | 1026 | 0.693 | −0.327 |
| *Caulleriella* sp. 1 | P | 536 | −0.428 | 0.835 |
| *Eunice antennata* Savigny, 1820 | P | 269 | −0.712 | −0.971 |
| *Asychis* sp. 1 | P | 217 | 0.948 | −1.011 |
| *Dimorphostylis cottoni* Hale, 1936 | C | 199 | −0.561 | 0.129 |
| *Euphilomedes* sp. 1 | O | 182 | 0.239 | 0.623 |
| *Exoediceros* sp. 1 | A | 177 | 0.102 | −0.398 |
| *Afromysis australiensis* Tattersall, 1940 | M | 146 | 0.058 | −0.560 |
| *Limnoporeia* sp. 2 | A | 138 | 0.056 | −0.390 |
| *Notospisula trigonella* (Lamarck, 1818) | B | 131 | 0.298 | 0.531 |
| *Magelona* sp. 1 | P | 114 | 0.584 | −0.048 |
| *Cirolana woodjonesi* Hale, 1924 | I | 97 | 0.162 | 0.445 |
| *Gastrosaccus dakini* Tattersall, 1940 | M | 92 | 0.025 | 0.334 |
| Oedicerotid sp. 4 | A | 72 | 0.788 | 0.428 |
| *Glyphocuma bakeri* Hale, 1936 | C | 71 | 0.017 | −0.151 |
| *Paraphoxus* sp. 9 | A | 64 | 0.407 | 0.241 |
| *Oediceroides* sp. 1 | A | 50 | −1.049 | −0.317 |
| *Ophiodromus* sp. 1 | P | 48 | −0.276 | −0.481 |
| *Dorvillea australiensis* (McIntosh, 1885) | P | 40 | 0.988 | 0.050 |
| *Paraphoxus* sp. 6 | A | 39 | 0.200 | 0.349 |
| *Rutiderma* sp. 1 | O | 39 | −0.349 | −0.228 |

could the pattern of stations in the two-dimensional space (defined by the first two canonical variates) be transposed to a map of the stations in real space such that a trend or zones were apparent.

In the third data reduction scheme (Table I) CV1 was dominated by a single environmental feature, the percentage of fine sand, which decreased with increasing distance from shore. Within the 1-m depth contour the percentage of fine sand ranged from 62 to 81% but beyond this line values from 2 to 62% were found. *Dorvillea*, *Asychis*, and oedicerotid sp. 4 were all negatively correlated with fine sand and were found only beyond the 1-m contour. *Oediceroides* and *Eunice* were also

negatively correlated with fine sand and distributed similarly to the other three so their loadings on variate 1 seem anomalous.

No environmental variables had particularly high loadings on CV2. The three species with high loadings on CV2 were most common at middle to greater depths.

Nevertheless, the station scores plotted for a canonical variate from each pair (Fig. 2) fell into discrete groups which were meaningful in real space (Fig. 3). Group

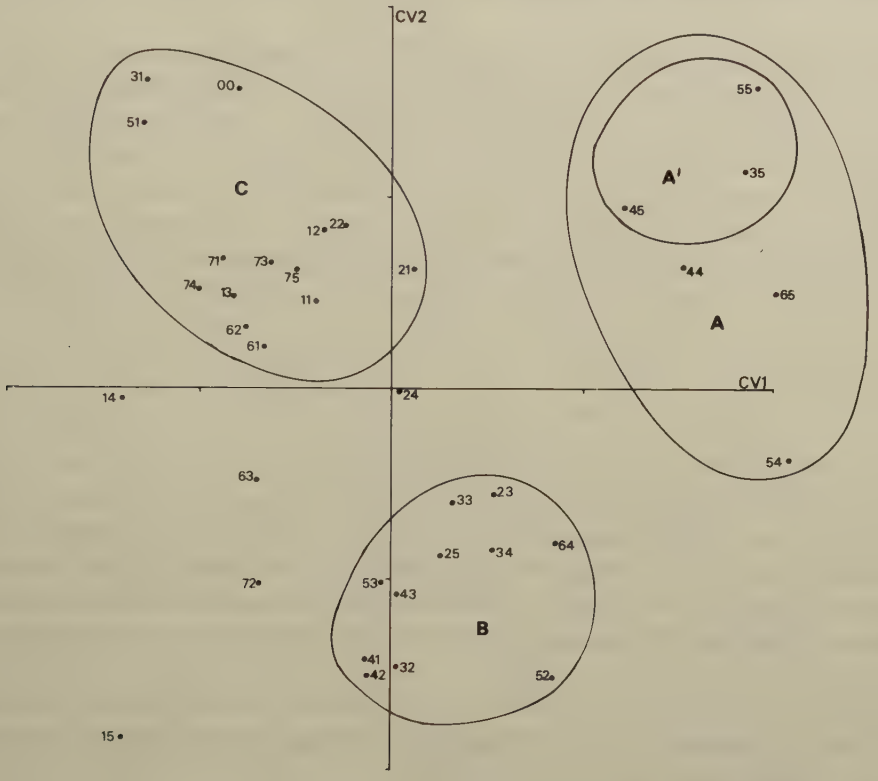


Fig. 2. Sampling stations plotted using scores on canonical variates 1 and 2: data reduction scheme 3; groups of stations obvious by inspection are circled and labelled A, A′, B, and C.

A, with highest positive scores on CV1, included deepest stations. Within this Group A′ comprised the three deepest stations with highest scores on CV2. Stations in Group B had intermediate scores on CV1 and low scores on CV2 and occurred at intermediate depths (Fig. 3). The remaining Group C included most of the stations within the 1-m contour (Fig. 3). Stations 14 and 15 had much lower scores on CV2 than expected. By examination of the original data it could be seen that the percentage of medium sand was greater here than at other stations in Group C. Percentage medium sand was one of the factors associated with CV2. The position of Station 72 cannot be interpreted in this way.

As the plot of scores gave meaningful results, the analysis was extended by computing the correlations between the variables (environmental and species) and the canonical variates. In the first instance this was done for the first two canonical variates and, when these were found to be encouraging, for the third variate as well.

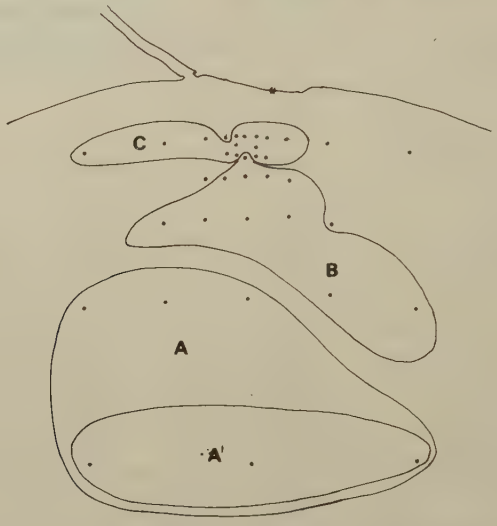


Fig. 3. Station groups (A, A′, B, and C derived from Fig. 2) plotted on the original sampling grid shown in Fig. 1.

The first canonical variate had strong correlations with most environmental variables. High positive correlations were found with silt, depth, and clay and high negative correlation with fine sand (Table II). The variate can therefore be said to represent a gradient of increasing depth and associated changes in sediment characteristics.

Correlations with CV2 were weaker but a positive correlation with phosphate and negative correlations with medium sand and carbonate were apparent (Table II).

TABLE II

Correlations between variables and the first three canonical variates obtained using data reduction scheme 3.

| Variable | CV1 | CV2 | CV3 |
|---|---|---|---|
| Depth | 0.867 | −0.012 | −0.427 |
| Orthophosphate | −0.512 | 0.537 | 0.423 |
| Ammonia | −0.358 | −0.105 | 0.201 |
| Percentage carbonate | 0.459 | −0.667 | −0.169 |
| Sorting coefficient | 0.689 | −0.301 | 0.482 |
| Percentage medium sand | 0.262 | −0.723 | −0.035 |
| Percentage fine sand | −0.827 | 0.409 | −0.153 |
| Percentage silt | 0.890 | −0.122 | 0.298 |
| Percentage clay | 0.738 | −0.051 | 0.541 |

The variate is probably best interpreted as having a depth-related component and a component due to influence of nutrients from the sewage-farm drain.

Percentage clay and sorting coefficient had highest positive correlations with CV3 and depth highest negative correlation. These correlations seem contradictory in that greatest concentrations of clay and high sorting coefficient (poor sediment sorting) are expected at greatest depth. The variate would seem to indicate a contrary trend or patchiness in sediment characteristics different from that normally expected with depth.

Interpretation of correlations between species and canonical variates was made easier by plotting species in the variate-space. Species arranged themselves into two distinct groups when plotted against canonical variates 1 and 2, a group of six species with negative correlations with CV1 and positive correlations with CV2, and a group of 16 species with correlations opposite in sign. The symmetry of this arrangement suggests CV1 and CV2 are similar in grouping the species. A similar division appears when species are plotted against CV1 and CV3 but the 16 species in the second group are spread along the axis of the third variate (Fig. 4).

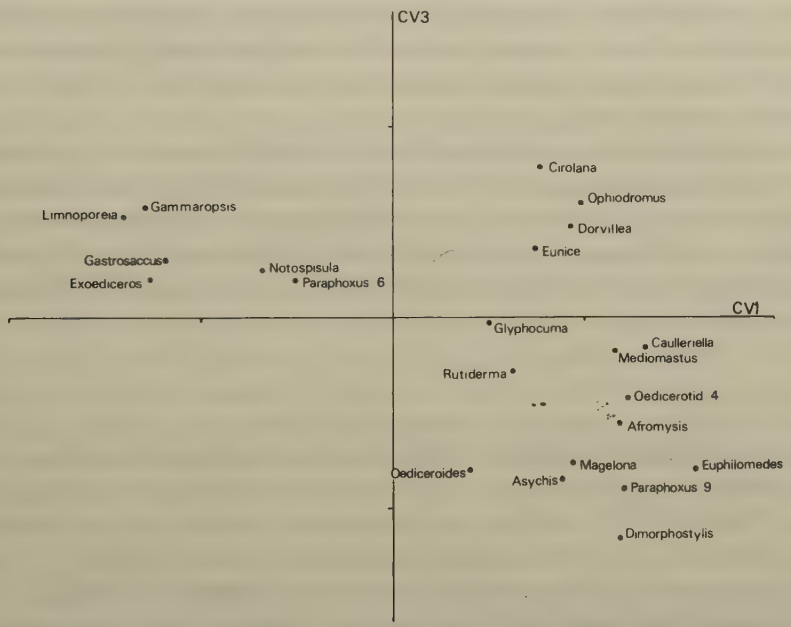


Fig. 4. Species plotted using correlations on canonical variates 1 and 3: data reduction scheme 3.

## Discussion

### Methods of data reduction

Of the several methods of data reduction, discarding the rarest species has met with few objections because, it is argued, the probability of co-occurrence of rare

species in any sample is small and is more likely due to chance than to similar habitat requirements. Elimination of rare species in fact has an effect similar to that of reduction in sampling effort. We reduced our original species list of 246 to 49 by excluding the rarest species. A further reduction by the same exclusion principle produced answers for the canonical correlation analysis which were difficult to interpret.

The second data reduction scheme eliminates species occurring at fewest stations. This has the disadvantage of removing from the analysis species confined to a single station or to a small group of stations. We suspected in this study that the sewage-farm drain would influence only a few stations. Removal of species confined to these stations defeats the purpose of the investigation and, as expected, gave unsatisfactory results in the canonical correlation analysis.

The third data reduction scheme gave the most easily interpretable results. Variables which do not correlate with each other (or with other variable-sets which are themselves interrelated) behave independently, unconnected with patterns elsewhere in the system. In the search for pattern they may as well be omitted. In addition, this scheme is less arbitrary, depending on examination of data relationships rather than restriction of the community studied. That is, the scheme does not violate the concept of a community as much as the other two schemes and provided us with ecologically sensible results.

Alternative data reduction schemes have been used in canonical correlation analysis. For example, Barkham & Norris (1970) replaced their original data matrix by a smaller matrix of eigenvectors from which those vectors associated with a small eigenvalue were omitted. The loadings of the original variables on the canonical variates can then be derived indirectly but Barkham & Norris were not satisfied with their analytical results.

## DATA ANALYSIS

There are at least three ways of giving the canonical variates meaning. One is to look at relative sizes of loadings, another is to plot station scores on the canonical variate. The third is to look at the correlations between original variables and canonical variates. The first approach appears to be unsatisfactory. The loadings are not independent of one another, so removing a particular variable from an analysis causes the loadings of the other variables to change magnitude. It was found that removing a species at random from those used in the third data reduction scheme (thus removing thirty correlation coefficients) changed the loadings reported in Table I out of all recognition.

The use of station canonical scores was a valuable aid to describing our results and relating them to sampling space (see Figs. 2 and 3). Surprisingly, this approach appears not to have been used before in the ecological literature. This is probably because scores are of little value in the sociological problems for which canonical

correlation analysis was developed. An anonymous individual's score on a canonical variate is of no value to the sociologist but in ecology it is possible to relate scores to an extrinsic variable, the stations' spatial relationship to each other.

Computing the correlations between the original variables and their corresponding canonical variates enables us to do two things: first, group together those species with high correlations with some canonical variate, and secondly take the environmental variables correlating highly with the other canonical variate of its pair to explain the factors causing this grouping.

The choice of how many canonical variate pairs to include in the results can be made on the basis of significance tests on the canonical correlation coefficients. The size of the canonical correlation coefficient is dependent upon the relative number of stations and data variables. Hence a value very close to unity may look impressive but, as in the above reduction schemes, is only high because the number of stations is only one or two above the number of data variables. Tests of hypotheses concerning the significance of canonical correlation coefficients are obviously irrelevant if the coefficient is unity. For following pairs of canonical variates they are valid only if the stations are a random sample of the area of interest, which is not the case for transect sampling. Using the significance level produced, however, by the SPSS package as a guide only, the first two or three pairs of canonical variates in each of our data reduction schemes were retained for further analysis.

Geometrically, a canonical correlation of unity implies that the position of the stations on a line in the species-space is identical to the positioning in the environmental-space, apart from a multiplying factor. This does not mean that these canonical variates are important factors in the original spaces. A better measure of the way in which species distributions are determined by the environmental variables measured may be the redundancy (Cooley & Lohnes, 1971, p. 171). In this study, for the first three canonical variate pairs, 48% of the variation in the species was associated with variation in the environmental variables. Total redundancy is analogous to the squared multiple correlation coefficient in multiple regression analysis and in fact canonical correlation analysis reduces to regression analysis when either $p$ or $q$ equals one.

## RESULTS OF THIS STUDY

It certainly aids in interpretation if the environmental canonical variates can be explained in terms of few variables as was the case with the third scheme. It was a fault of the first two schemes that several environmental variables had high or similar loadings on the variates. On the other hand, the ecologist wanting to explain the biotic attributes in terms of the abiotic attributes is delighted if several species have high loadings or correlations on a variate explained by a single abiotic variable.

In this study, CV1 represented a gradient of depth and associated sediment properties, CV2 represented depth and nutrients, and CV3 represented changes in sediment

characteristics not associated with depth, i.e. patchiness in the abiotic habitat.

The clear division of the species into two groups along the first three canonical variates enabled characteristic ("indicator") species to be recognized. In Fig. 5 these groups were compared with the six species-groups obtained by classification analysis by Poore & Kudenov (1978, their Table 3). (Note that the species used in the canonical correlation analysis were only a subset of those used in the classification.)

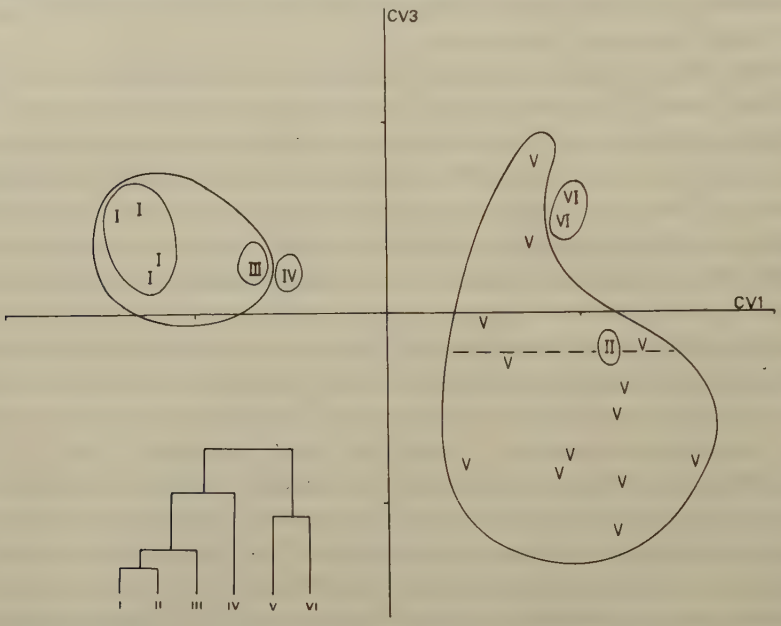


Fig. 5. Species plotted using correlations on canonical variates 1 and 3 (Fig. 4): species are here labelled with the group (I to VI) to which they were assigned in Poore & Kudenov's classification; (1978, their Table 3); the hierarchy of groups (inset) is given and translated to the plot by encircling progressively higher levels in the hierarchy; the position of Group II is anomalous (see text); the dashed line divides sub-groups of Group V; *Dorvillea* was incorrectly given as cf. *Synasterope* sp. 2 by Poore & Kudenov (1978, their Table 3).

The species from Groups I, III, and IV (together combined at higher levels in the hierarchy) were confined to or most dense at shallowest stations. Species in Groups V and VI (the second half of the hierarchy; Fig. 5) were most abundant in deep water. *Mediomastus* (from Group II) was mis-classified in Poore & Kudenov's treatment and is better placed with deep-water species as happens in the canonical correlation analysis.

Species Group V contained the largest number of species and was relatively confined on CV1 and CV2 but had both positive and negative correlations with CV3 (Fig. 5). Subsequent examination of the hierarchical classification indicated that this group can be further divided into two sub-groups, the first of species whose correlations with CV3 were $> -0.1$ and the second of species whose correlations

with CV3 were $< -0.1$ as indicated by the dashed line in Fig. 5. The species with highest positive correlations (*Dimorphostylis*, *Paraphoxus* sp. 9) were confined to stations at depths >2 m. The species with highest negative correlation (e.g. *Cirolana*) were more scattered and more widespread.

## CONCLUSION

The success of this study suggests that canonical correlation analysis has not received the attention in benthic ecology that it deserves. We have shown how it is possible to erect station groupings, define characteristic species for different habitats and to relate the distribution of species and samples to environmental variables. More popular classification techniques fail to meet the last, and often most important, of these objectives.

It is almost axiomatic that the structure and function of the benthos is determined in the first instance by the nature of sediment. Thorson's (1957) concept of parallel level-bottom communities relies on this proposition but there has been very little research on the particular features of the sediment to which animals respond. Sanders (1958) found that the density of macrobenthos correlated with the percentage of clay, but suggested that high levels of organic matter associated with clay were responsible for the nature of clay-loving fauna. On a gross scale the sediment-fauna relation is probably true, but what rôle do grain size and other sedimentary properties play in determination of patchiness or trends in species distributions over intermediate or small scales? Are their effects masked by interspecific competition? Lie (1974) used principal components analysis of a benthic fauna followed by multiple regression to explore the relations among the extracted components and environmental variables. A correlation was found between one of the components and mean particle size which was independent of depth. Smith & Greene (1976) used similar methods with some success for benthos influenced by a submarine outfall. Few authors have, however, attempted to demonstrate such a statistical relation between biotic and abiotic variables on small scales and use of canonical correlation analysis for this purpose seems well worthwhile.

## Acknowledgements

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# WATER RELATIONS AND PHYSIOLOGICAL ECOLOGY OF THE SALT MARSH SNAIL, *MELAMPUS BIDENTATUS* Say

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**Abstract:** Field and laboratory studies were conducted on the water relations of the high intertidal snail *Melampus bidentatus* Say (Pulmonata : Ellobiidae) in the Little Sippewisset Marsh, Cape Cod (Mass., U.S.A.). Densities of *Melampus* in the upper 12% of the intertidal zone averaged about 1300 snails/$m^2$. A size gradient of snails was found, with large snails (5–12 mm shell length) occupying both upper and lower extremes of tidal height. Adult snails ( >5 mm) undergo semilunar migrations, such that during neap tidal cycles the center of peak snail density shifts 2 m lower in the marsh from that of spring tidal cycles. Juvenile snails appear to remain in the dense vegetation of mid-range.

During dry conditions in the field, snails contained 15% less body water compared with normally hydrated snails. Laboratory studies on desiccation (controlled temperature and humidity, still air) showed water loss to be inversely proportional to shell length and relative humidity (RH). Immature snails regulate water loss poorly; few survive for more than 12 h in 0–75% RH (20 °C). In contrast, adults survive for 48 h or more in ≤50% RH and remained alive in 97% RH for at least 14 days, despite the loss of up to 40% body water. Adult snails placed in ≈0% RH for 27–36 h were remarkably tolerant to desiccation, with an $LD_{50}$ near 80% body water loss. *Melampus* rehydrates rapidly when provided with free water; from a level of 50% body water loss, snails reached 90% normal water content within 0.75 h. Adult *Melampus* can survive submergence in sea water for at least 10 days and in fresh water for 3 days and can tolerate −12 °C for several days.

These studies demonstrate some of the physiological and behavioral adaptations to the rhythmicity of the tides and to the extremes in other physical factors found in the high intertidal zone. The significance of these adaptations in *Melampus*, and the fitting of its primitive, marine reproductive strategy to an amphibious lifestyle, are discussed in relation to the transitional status of this snail in the movement of molluscs from marine origins to land and freshwater habitats.

## INTRODUCTION

The amphibious, air-breathing snails of the genus *Melampus* are among the most abundant animals of the high intertidal zone of salt marshes along the Atlantic coast. As members of the family Ellobiidae, they are likely to be closely related to an ancestral marine group that became established in the intertidal habitat and gave rise to groups that colonized first land and then freshwater habitats (Morton, 1955; Russell-Hunter *et al.*, 1972; Russell-Hunter, 1978, 1979).

The extreme ranges and variability of physical factors (tides, temperatures, salinity, etc.) in marsh areas of estuaries and the high intertidal zone demand

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special adaptations of animals residing there. The highly successful adaptation by *Melampus* of its reproductive strategy (despite the primitive conditions of large numbers of eggs and planktonic larvae) to a primarily terrestrial existence has been well documented by Russell-Hunter and his colleagues (Apley, 1970; Russell-Hunter *et al.*, 1972; Price, 1979). Except for abstracts on the respiration of *Melampus* (McMahon & Russell-Hunter, 1975) and on its water loss (Russell-Hunter & Meadows, 1965), few other aspects of the success of *Melampus* in the intertidal habitat have been studied.

The objectives of the present work were to study the behavioral and physiological adaptations of *Melampus* to the wide range of water conditions found in the high intertidal zone. Of special interest were water losses actually experienced by field animals and the specific means of reducing and tolerating such stress. A preliminary note on some of this work has appeared (Price & Russell-Hunter, 1975).

## Materials and Methods

Field studies were conducted on a population of *Melampus* in the Little Sippewisset (LS) marsh, Falmouth (Mass., U.S.A.), with much of the work performed in a marked 50 × 10 m area along the southern border of the marsh. The vertical vegetation zones (Fig. 1) in this area are well defined and typical of New England salt marshes (Chapman, 1938; Redfield, 1972), although the LS marsh is much less extended horizontally than many other marshes in Massachusetts. Measurements of tides and temperature were recorded on site; other weather data were obtained from Otis Air Force Base Weather Station, Cape Cod, and from the Woods Hole Oceanographic Institution.

Population distributions, size gradients, and migrations were studied in field plots of known tidal level in the intertidal zone. Transect lines 9.0 m long were set out at right angles to the shore line, from a point at about mean high water down to the middle of the tall *Spartina alterniflora* zone (Fig. 1). A wooden frame 30 × 30 cm was positioned over even-numbered plots (i.e., every other 30 cm) along the transect line. Larger snails contained therein were counted and measured. One quarter (225 $cm^2$) of the plot's grass/soil surface was removed (replaced later) to the laboratory for counting small snails. Six transects, each at least 1 m away from all others, were studied on days selected for contrasting tidal heights.

Snails collected for laboratory study were maintained (for at least 1 wk prior to experiments) in glass-topped bowls which were lined with filter paper soaked with 75% sea water. Snails were fed dried lettuce. Shell lengths (from the spiral apex to the distal lip of the shell aperture) were measured on larger snails to 0.1 mm with vernier calipers. Snails under 4 mm were measured to within 0.5 mm with a metric ruler under a dissecting microscope. Weights were determined on torsion balances with resolutions of 0.05 mg for larger snails or 5.0 $\mu$g for smaller snails.

Normal "weight wet" refers to weight of fully hydrated snails with shells dried and cleaned; animals were weighed after gentle stimulation to cause retraction and expulsion of pallial water (such pallial water can be significant, ranging from 3 to 13% of "wet tissue weight" according to Blinn, 1964). "Dry weight" is the weight of a whole animal after death and drying at 105 °C for 5 h to constant weight. "Body water" is the difference between these first two measures. "Shell weight" was determined by acid digestion (12% $HNO_3$ for 5 h) of a pre-weighed dried whole snail. Tissue remaining after such acid digestion was dried again and is "tissue dry weight".

Glass desiccators served as closed, controlled humidity chambers; these were partially filled with solutions of water and sulfuric acid to establish relative humidities of from near 0% to near 95% (Solomon, 1951). Chamber humidities were checked with a hair hygrometer. Desiccators were kept in constant fluorescent light in environmental chambers maintained within $\pm$ 0.5 °C. Pre-weighed and measured snails were held in dry, 9 cm Petri dishes covered with wire-mesh nylon screening and placed in the desiccators.

Water loss was monitored by weighings at 2-h intervals over the first 12 h and at longer intervals thereafter. Dry weights and amounts of total body water were obtained for all animals at the end of each experiment. Rates of rehydration were determined by placing partially desiccated snails on well-soaked filter paper and weighing at $\approx$ 10-min intervals.

Size-class groups of 50 snails were desiccated (at 0% RH) and random samples of 5–10 animals were removed periodically to estimate size-class dependent mortality. One size class (8.5–10 mm) of snails was used to determine the absolute limits of desiccation tolerance. Weights of marked individuals were monitored and when a desired percentage of body water loss was reached, the snail would then be allowed to rehydrate. The effect of aggregation on water loss was investigated using two groups of 70 animals, each in separate wax-lined Petri dishes, one of which was subdivided with short pins into 35 separate cages holding two animals each. About once per hour, each group was removed and weighed in a tared Petri dish on a Mettler pan balance to 0.1 mg.

These weighing and survivorship methods were also applied to weight changes and survival of animals submerged (for 1–12 days) in liter quantities of dechlorinated tap water or 100% sea water kept at room temperature (19–21 °C).

A "normal wet weight" relationship with shell length was derived from freshly collected animals (minimum of five for each 0.2-mm interval from 6.0 to 10.0 mm shell length) maintained on moist filter paper for 2 days. Weighings were made twice each day over 4 days and a mean wet weight was computed for each snail.

## Results

### PHYSICAL FACTORS AT STUDY SITE

With little demonstrable pressure from the biotic environment, either predatory (except for the smallest individuals; Vince *et al.*, 1976) or competitive (Price, 1976), the dominant feature in the ecology of *Melampus* at this study site is clearly vertical zonation. Russell-Hunter *et al.* (1972) determined that populations of *Melampus*, living in the upper 12% of the intertidal zone in LS marsh, are bathed by sea water only from 2.3 to 4.0% of each month's time. These estimates were confirmed in the present work and show that *Melampus* is fundamentally a terrestrial animal, bathed infrequently but regularly by the spring tides of the semilunar cycles.

During the period of this study, the LS marsh area had an average of 9 days per month with significant precipitation (Cape Cod receives about 28 in/yr; Redfield, 1972). During heavy rainfalls, *Uca* crab burrows fill rapidly and water on the marsh surface is presumably of lowered salinity. Thus, *Melampus* populations, especially those estivating in *Uca* burrows in winter (Apley, 1970; Price, 1976), are subjected to periods of potential osmotic stress, from the high salinity tidal water to low salinity fresh water from precipitation.

Temperatures at the LS marsh site (at ground level) range from about $-12\,^{\circ}C$ in winter to over $40\,^{\circ}C$ occasionally in summer. Such extremes over short periods are probably not very stressful for *Melampus* (McMahon & Russell-Hunter, 1975, 1977) but high temperatures could be when combined with the long, dry periods during neap tidal cycles when the *Melampus* zone remains unwetted.

### DISTRIBUTIONS, SIZE GRADIENTS, AND MIGRATIONS

The densities of *Melampus* in relation to tidal height and vegetation zone are shown in Fig. 1. Snails are generally absent from the marsh surface from November to March, but can be found in crab burrows and under stones. Densities of *Melampus* generally range from 5 to 20 snails per 100 $cm^2$; in the most highly populated areas of the LS marsh, the overall average is about 1300 snails per $m^2$. Densities of *Melampus* in other salt marshes in Massachusetts do not appear to exceed this figure. Large increases in population numbers occur, however, after spat falls in mid- to late summer as first observed by Russell-Hunter *et al.* (1972). Thus, in the Great Sippewisset Marsh, Vince *et al.* (1976) recorded densities of newly settled *Melampus* of up to 5000/$m^2$, numbers which undoubtedly decline rapidly due to predation and other pressures. Densities fall off rapidly in both the onshore and offshore directions from the peak numbers in the *Distichlis* and upper, short *Spartina* zones (Fig. 1). Snails are rarely found in either the dry supralittoral shrub zone or the sandy drainage channels.

The extremes of the intertidal range of the *Melampus* distribution are dominated by larger snails (Fig. 2), while smaller, newly-settled snails remain largely in the dense vegetation of the short *Spartina* zone, which receives the "average" amounts of both inundation by flood waters and exposure to air. This area is also richest in food for *Melampus*, which includes decaying surface litter and grasses and the Aufwuchs (algal colonies) that grow upon such litter.

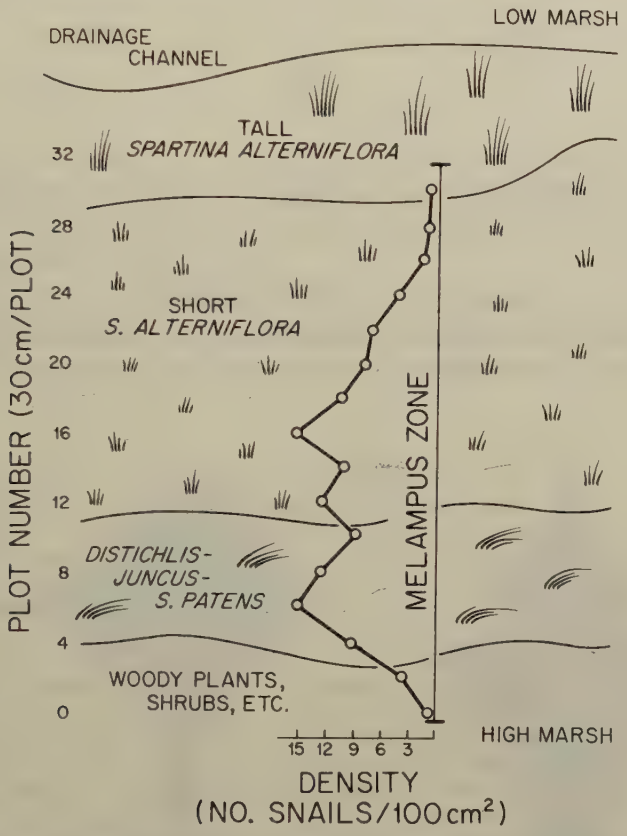


Fig. 1. Sketch of marsh study site: vegetation zones and the distribution and densities of *Melampus* are shown in relation to tidal height (plot numbers from high to low marsh); densities of snails are means per 100 $cm^2$ of marsh surface and were derived from counts of all snails in 900-$cm^2$ plots along transect lines (see text for details).

The combined effects of high air temperatures, low humidities, and neap tidal cycles in the marsh result in occasional periods of dryness for *Melampus*. Weights of snails collected from the field on days of dry conditions show significant departures from weights expected of fully-hydrated snails (Tables I and II). Expected weights were generated from a regression of normal wet weights of fully-hydrated, measured snails: weight (mg) = $-173.1 + 34.2 \times$ shell length (mm); $r = +0.993$. As shown in Table I, the average snail collected during hot dry periods

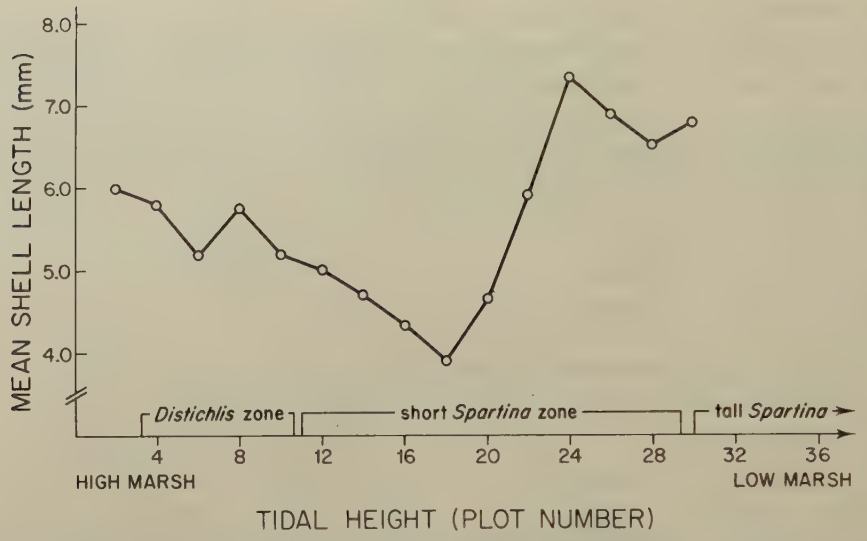


Fig. 2. Size gradient of *Melampus* in relation to tidal height: mean shell length derived from measurements of snails ( >1 mm shell length) present in transect plots used for density measurements in Fig. 1.

TABLE I

Weight loss in adult *Melampus* in the field.

| | | | | Mean weight disparities† | | | |
|---|---|---|---|---|---|---|---|
| | | | | As collected | | Rehydrated** | |
| Collection | Date | *n* | Field conditions* | Mean | Range | Mean | Range |
| 1 | 30 July | 46 | Neaps, 35 °C, low RH, dry | −8.2 ± 1.1 | −22.6 to +8.1 | −2.2 ± 1.0 | −18.5 to +9.5 |
| 2 | 31 July | 30 | Neaps, 36 °C, low RH, dry | −8.0 ± 1.0 | −19.8 to +4.5 | −0.7 ± 0.8 | −11.6 to +7.8 |
| 3 | 2 August | 46 | Neaps, 32 °C, low RH, dry | −7.9 ± 1.2 | −17.8 to +14.9 | — | — |
| 4 | 27 May | 46 | Springs, 24 °C, high RH, wet | −2.1 ± 1.2 | −16.2 to +12.1 | — | — |
| 5 | 4 June | 32 | Springs, 26 °C, high RH, wet | −1.2 ± 1.3 | −13.8 to +11.1 | — | — |

† Weight disparity is a mean percentage departure (+ or −) from expected (full hydration) weights generated from a regression equation of normal snail weights on shell length (see text for details). Percentages ± SEM.

* Field conditions include tidal cycle (neaps or springs); maximum air temperature for day; relative humidity for day; wetness (subjective) of marsh soil (wet or dry).

** Animals allowed access to water for 24 h, then reweighed.

TABLE II

Selected $t$-test comparison of field weight loss data (Table I): value $\hat{t}$ is computed; appropriate table value of Student's $t = 2.576$ ($\alpha = 0.005$, d.f. $= \infty$).

| Collection comparison | d.f. | $\hat{t}$ value | Significance |
|---|---|---|---|
| 1 and rehydrated 1 | 90 | 4.710 | <0.005 |
| 2 and rehydrated 2 | 58 | 5.508 | <0.005 |
| 3 and 4 | 90 | 3.787 | <0.005 |
| 1 rehydrated and 4 | 90 | 0.365 | n.s. |
| 1 rehydr. and 2 rehydr. | 74 | 0.467 | n.s. |

weighed about 8% less than expected. This difference represents a loss of about 15% of total body water, a condition reached within 8 h in laboratory snails placed in 97% RH at 20 °C and deprived of free water. A few individuals in the field lost as much as 40% of body water.

Preliminary observations suggested that, at least in spring and early summer, snails migrated up and back on the shore in phase with neap and spring tidal cycles, and quantitative studies confirmed this phenomenon (Fig. 3). The position

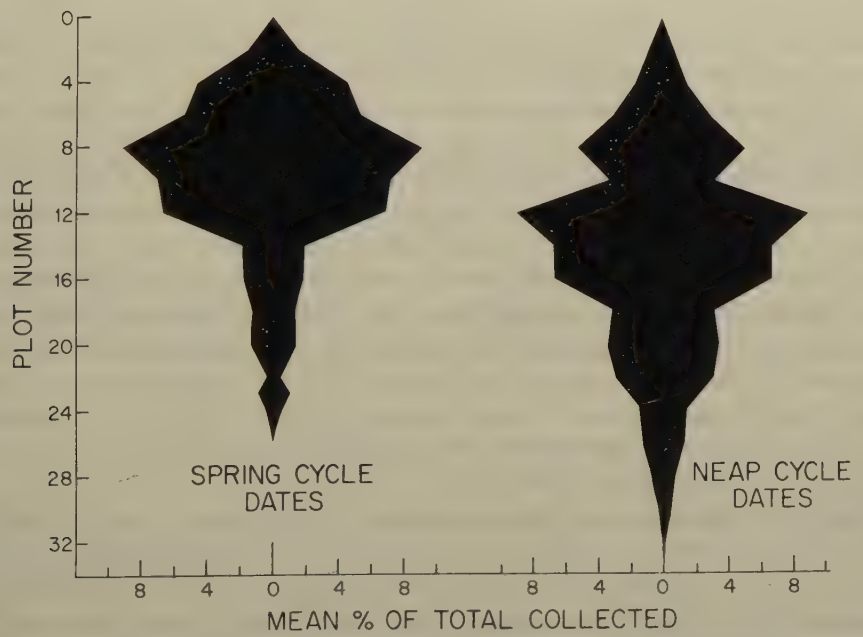


Fig. 3. Semilunar migrations of adult snails within the upper intertidal zone: six separate transect lines (at right angles to the shore line) were established after high tides (p.m.) on 6 days (3 during the spring tidal cycles and 3 during the neap cycle during spring and early summer); all snails >5 mm shell length were counted in each of the even-numbered plots (see Fig. 1); note that the bulk of the population shifts its position toward lower marsh levels during neap tidal cycle.

of peak densities along the transect lines occurred ≈ 2 m lower (horizontal distance in an off-shore direction) during neap tidal cycles compared with spring cycles. Since small snails ( <5 mm) did not appear to participate in this migration, shifts in densities were also estimated by counting the numbers of large snails present in the *Distichlis* zone. There was a strong correlation between numbers of large snails present in this zone at certain tidal heights on 6 measurement days (3 during spring, 3 during neap tidal cycles): % of large snails in *Distichlis* zone = $-136.5 + 18.3 \times$ tidal height (ft); $r = +0.931$.

## EXPERIMENTS ON DEHYDRATION STRESS

In the absence of free water, at humidities from 0–97% (20 °C), snails lost weight in the experiments reported below. At humidities near 95%, one-third of body water was lost from adult snails before the saturation deficiency at the skin was lowered to a level at which further evaporation took place at only a nominal rate. For reference purposes, the following weights of body parts in *Melampus* are provided. For adult snails (5.0 to 11.5 mm shell length): shell weight, 37.8 ± 1.4% (SD); body water, 50.4 ± 4.0%; and tissue dry weight, 12.6 ± 2.7%. For juvenile snails ( <5.0 mm): shell weight, 31.1 ± 0.3%; body water 57.6 ± 3.9%; and tissue dry weight, 12.4 ± 3.1%.

Desiccation (at 0% RH, 20 °C, 80 h) of small groups of snails in several size classes showed that larger snails remained active and lived longer than did smaller snails. An $LD_{50}$ measure was established as the point at which 5 of 10 sampled snails failed to recover from a particular treatment (i.e., remained inactive 48 h after returning them to sea-water-soaked filter paper). Survival of snails in this size-class experiment was found to be closely related to shell length $LD_{50}$ (in hours of desiccation) = 3.06 + 8.07 × shell length (mm); $r = +0.865$. Furthermore, large snails tended to aggregate during the desiccation experiment while smaller snails rarely did so. When equal numbers (70) of large snails were desiccated either as an aggregated group or as separate individuals, snails in the aggregated group lost an average of 25% less water per hour (over the first 20 h) than did the average separated snail. This difference in rate of water loss was negligible after 20 h, the time at which individuals in the group allowed to aggregate did not do so; they remained immobile and withdrew into their shells.

Experimental results on the interrelationships of size, relative humidity, and rates of water loss in individual snails are shown in Figs. 4–6. At ≈0% RH and 50% RH, few snails of any size survived more than 48 h (20 °C). At higher humidities, larger snails can live for many days (at least 14 days in 97% RH) in the absence of free water, whereas small snails generally live less than 12 h without free water.

Rates of water loss in adult snails, expressed in mg $H_2O$ lost per mg tissue dry weight per hour, are inversely related to RH (Fig. 4); in all but 0% RH, deceleration of those rates over time is relatively constant. Water loss in juveniles was so rapid

and rates so variable that no meaningful regressions could be calculated. The time course of body water loss, expressed as a percentage of total body water, for this size class is obviously different from that of the adults (Fig. 5). For both adults and juveniles, the time-course curves (Fig. 5) for 97% RH show minimal weight losses from 10 and 40 h onward, respectively, and probably reflect near-equilibrium saturation deficiencies between tissue and atmosphere.

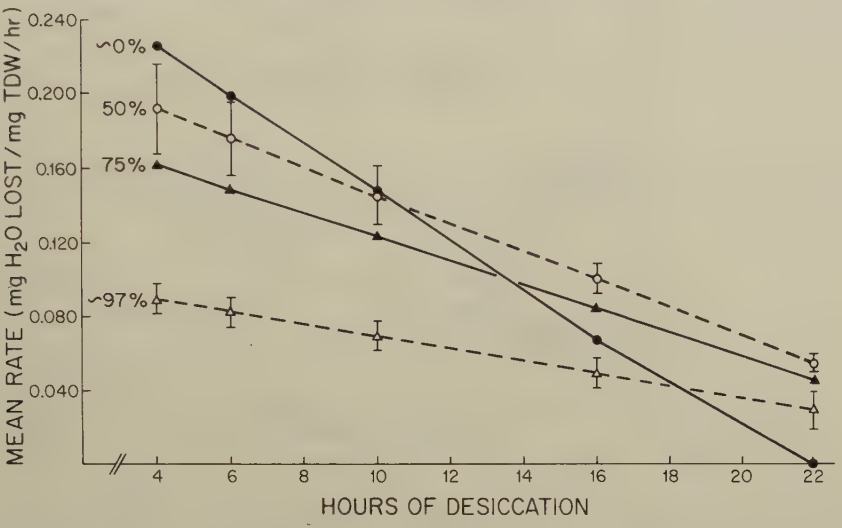


Fig. 4. Rates of water loss in adult snails over 24 h at four relative humidities (0, 50, 75, and 97% relative humidity; still air; 20 °C): each point is a mean rate for 12 animals (4 snails from 3 size classes: 6.2, 8.4, and 10.7 mm shell lengths); standard errors of the mean are shown for the 97 and 50% RH experiments (rates in the 50% RH experiments showed the greatest variability of all experiments); water loss is expressed as mg $H_2O$ lost per mg tissue dry weight (TDW) per hour; lines fitted by eye.

Large snails exhibit a remarkable degree of tolerance of water loss, as was demonstrated using snails (8.5 to 10.5 mm) desiccated at 0% RH over 27–36 h. The level of $LD_{50}$ (defined above) was determined as the loss of 78.5% of body water

TABLE III
Tolerance of adult *Melampus* to desiccation.

| Percentage body water lost* | Approximate mg $H_2O$/mg tissue dry wt. | *n* | Survived** | Percentage mortality |
|---|---|---|---|---|
| 0 (full hydration) | 4.58 | — | — | — |
| 70–75.5 | 1.12 | 75 | 72 | 4.0 |
| 75.6–77.5 | 1.03 | 36 | 23 | 36.1 |
| 77.6–80 | 0.92 | 14 | 5 | 64.3 |
| <80 | <0.89 | 30 | 1 | 96.7 |

* Snails were placed in 0% RH for 27–36 h.
** Surviving snails were those alive and active 48 h after providing them with free water.

(Table III). As shown in column 2 of Table III, there can be an approximate fourfold reduction in mg $H_2O$ per mg tissue dry weight prior to death. After desiccation to about 75% body water loss, snails were usually active in less than 2 h after providing them with free water.

Water loss rates in adult snails (8–11 mm) were linear with respect to temperatures of 10, 20, and 30 °C, over 10-h periods of desiccation, at either ≈97% (correlation coefficient, $r = +0.996$) or 50% RH ($r = +0.992$).

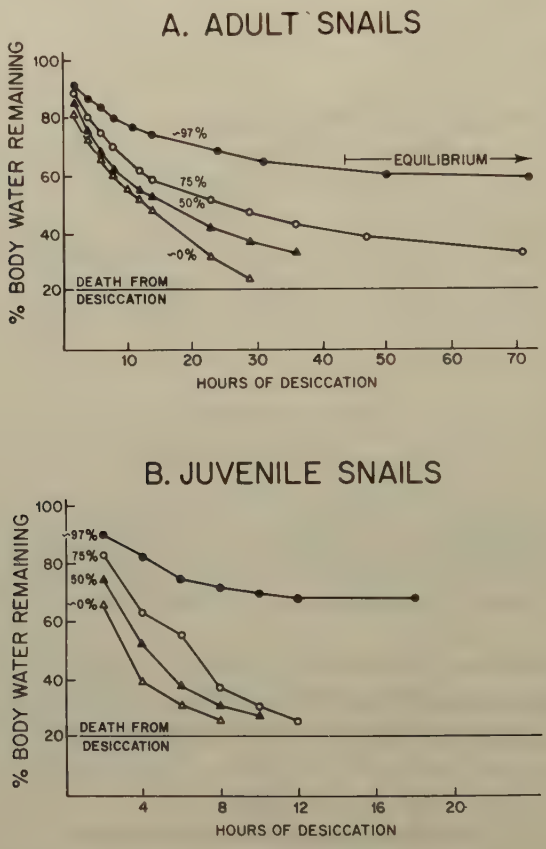


Fig. 5. Rates of water loss from juvenile and adult *Melampus* in relation to relative humidity: A, adult snails (8.4–10.7 mm shell length); desiccations carried out in still air at 20 ± 0.5 °C and at four relative humidities (in parentheses on graph); eight marked snails were used in each RH experiment; line ("death from desiccation") shows 80% body water loss which resulted in death for >95% of snails; "equilibrium" represents period (extended for several days) during which snails remained alive and lost little water; B, juvenile snails; experiments as in A, using 16 snails 3.0–4.0 mm; note change in time scale; most juvenile snails were near death within 12 h in each RH except 97%.

## REHYDRATION, SUBMERGENCE, AND FREEZING

Rehydration in dehydrated *Melampus* is rapid, as shown in Fig. 6. Two size classes of snails (5–6 mm and 8–9 mm shell length) were used and no difference between groups was found.

As mentioned earlier, tidal inundations or sustained rainfall can result in temporary submergence of *Melampus* in water of varied salinity. In an earlier study, McMahon & Russell-Hunter (1977) found that *Melampus* adults remained active, though lethargic, under 100% sea water (20 °C). Their respiration rates fell to about 10% of aerial rates at 20 °C. In the present work, adult snails were found to survive full

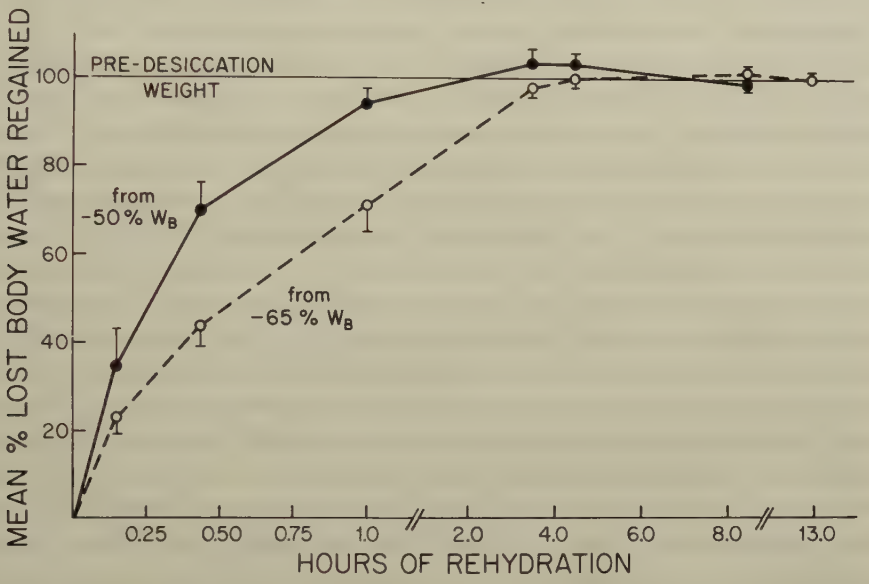


Fig. 6. Rehydration of desiccated snails: adult snails ($n = 40$; 5–12 mm shell length) were placed in ≈0% RH and desiccated for 10–20 h to either 50 or 65% body water loss (20 snails each); snails were then provided with free water and weighed during the course of rehydration; "predesiccation weight" represents 100% (normal) body water content for each snail.

submergence in sea water for at least 10 days (20 °C) and in tap water for up to 3 days. After 7 days in sea water, all snails are generally withdrawn, but most become active almost immediately on removal from the water. Submerged in tap water, snails become inactive after only a few hours and secrete copious amounts of mucus. Weight changes, followed for groups placed in sea or tap water (25 snails in each) were minimal: at 48 h, there was a loss of 5.33% of body water per snail in sea water and a mean gain in fresh water of 4.84%. Because considerable mucus was removed prior to weighing, the weight gains recorded in tap water were probably less than actual.

Larger *Melampus* tolerate freezing remarkably well. Overwintering adults from the field were placed in a laboratory freezer at −12 °C, and after 3 days, the

following mortality percentages were recorded: <5 mm, 85.7% (30 of 35 dead); 5–7 mm, 68.8% (11/16); and >7 mm, 26.7% (4/15). Ice crystals were clearly visible in the aperture of most snails (also observed in *Littorina* by Kanwisher, 1955). The swollen bodies of several of the large animals were apparently frozen solid, but more than 70% of these recovered.

## Discussion

The physical factors in the intertidal zone in which *Melampus* evolved have changed little over 180 million years (since the Jurassic; Harland *et al.*, 1964, 1967), although the existence and complexion of individual habitats, particularly salt marshes, have cycled much faster (Redfield, 1972). The need for discontinuous colonization and restoration of decimated and marginal stocks may well be among the significant reasons for the retention by *Melampus* of its primitive characteristic of producing massive numbers of motile larvae (as many as 35 000 per snail per year; Russell-Hunter *et al.*, 1972). The specific, functional use of the tides as a dispersing agent clearly and essentially ties this amphibious species to the upper intertidal zone. For most animals living in this area, the inundating periods of tidal cycles are of much less direct physiological importance than are the considerably longer periods of aerial exposure (Southward, 1958) which create, temporally and spatially, a basically terrestrial environment in the upper levels where *Melampus* lives. Homeostatic problems, with respect to the great range in variability of most physiochemical properties of the high intertidal zone, are well illustrated by those of water control. In the present study, *Melampus* was found to experience significant water loss in the field but it also was found to possess broad tolerances to widely variable water conditions.

Small, immature *Melampus* (<5 mm shell length) have little control over water loss, as reported for other molluscs (Schaefer *et al.*, 1968a, b; Davies, 1969; Cameron, 1970; Whitlach, 1972; Van der Laan, 1973). These juvenile *Melampus* remain in the more sheltered microclimates (under plants, rocks, and shells) within the middle portion of the overall distribution of *Melampus* (Fig. 1). This undoubtedly contributes to the observed size gradient of snails (Fig. 2), a phenomenon reported for other species of snails (reviewed by Vermeij, 1972; and see Chow, 1975). Such size gradients, as well as interspecific zonations in molluscs (Woodward, 1909; Mattox, 1949; Stephenson & Stephenson, 1949; Boyle, 1970), have been shown to be related to both differential desiccation rates (as in the present work and, for example, Kensler, 1967; Davies, 1969; Edwards, 1969; Newell, 1970) and respiratory capacities (e.g., Newell, 1970; McMahon & Russell-Hunter, 1977, 1978). Another factor which contributes to the size gradient of *Melampus* in the intertidal zone may be predation. Vince *et al.* (1976) report that the killifish *Fundulus* readily feeds on small *Melampus*, but rarely consumes snails >4 mm shell length. They

found that smaller *Melampus* preferentially settle and remain in the "high marsh" of the Great Sippewisset Marsh, where predation by *Fundulus* is reduced. Snails were generally larger at their "low marsh" sampling sites, in agreement with data presented here. They sampled only from these two sites and did not examine the size gradient distribution of snails in relation to all tidal heights. Thus, a real comparison of their findings with those reported here cannot be made.

Adult *Melampus* are found at the highest intertidal levels of the marsh, but semilunar migrations downshore apparently serve to reduce periods of potentially dehydrating exposure during periods of neap tides. As with size gradients, such population movements have been recorded for other molluscs, but most are annual rather than lunar-related migrations and are usually stimulated by shifts in temperature (in fresh water: Cheatum, 1934; Clampitt, 1974; Russell Hunter, 1953, 1978; in salt water: Magalhaes, 1948; Williams & Ellis, 1975). Only a few reports of semilunar migrations have been published for invertebrates (e.g., molluscs: Berry, 1975; crustaceans: Edney, 1957; Enright, 1963; review by Palmer, 1973). In contrast, there are several studies on twice-daily tidal migrations in invertebrates such as turbellarians (Gamble & Keeble, 1903) and diatoms (Fauré-Fremiet, 1951; Round & Palmer, 1966).

In dehydrating periods of elevated temperatures and lowered humidities, particularly during periods of low neap tides, the migratory behavior of *Melampus* is not sufficient to prevent water loss. Under such field conditions, the snails can experience significant water losses (up to 40% of body water in some cases). No other field measurements of this sort on ellobiid molluscs have been reported, and very few have appeared on other molluscs (Mead, 1961; Davies, 1969; Gilbertson, 1969; Courtney, 1972; Boss, 1974).

Other extremes in field water conditions for *Melampus* can include prolonged inundation with salt water (e.g., during highest spring tides, storms) as well as with fresh water (following heavy precipitation). *Melampus* apparently does not use its lung as a "physical gill" unlike some pulmonates (Russell-Hunter, 1953), but nevertheless can survive moderate periods (days) of total immersion in both salt and fresh waters presumably using cutaneous $O_2$ uptake at reduced rates (McMahon & Russell-Hunter, 1975, 1977; and the present work).

The more frequent and prolonged periods of dry conditions, however, pose a serious problem for *Melampus*, particularly for the adults that occupy higher, more exposed zones than do juveniles. Except at the highest humidity tested ($\approx$97% RH), juveniles lose water rapidly, apparently unable to exert much regulation. Adult snails, however, possess several means of reducing or tolerating water loss. The allometric characteristic of a decreased shell length: aperture opening ratio in larger *Melampus* could itself account for lower water loss rates compared with juveniles (Gebhardt-Dunkel, 1953; Schaefer *et al.*, 1968a, b). Similar reductions in water loss rates are accorded larger animals in a number of species, but the phenomenon is more usually a consequence of the animal's surface : volume ratio (Schmidt-

Nielsen, 1975). Such relationships in snails and bivalves are modified by the presence of their rather impermeable shell (Machin, 1976). Another physical means for reducing evaporative water loss in shelled snails such as *Melampus*, results from the behavioral "choice" (Russell-Hunter, 1979) of total withdrawal into the inner whorls of the shell, thus diminishing the amount of evaporative surface several-fold.

Detailed studies by Machin (1964, 1966) have shown that snails control water loss by physiological methods, and some regulation is also present in *Melampus*. The simple increase (up to 4 ×) in solute concentration in the body tissues of dehydrated *Melampus* can account for only a modest decrease in evaporative loss (Mellanby, 1935). *Melampus*, furthermore, does not form an epiphragm, which can be a moderately effective barrier to water loss (Machin, 1968; Boss, 1974). Without much contribution from physical factors, therefore, the steady decrease in the rate of water loss in dehydrating adult *Melampus* (Fig. 4) clearly suggests that some metabolic regulation occurs. Such physiological control may be exerted in part by the snail's integument. Machin (1974) has shown that the epidermis of *Helix* maintains a "waterproof" barrier, consisting of layers of increasing solute concentration towards its surface, a process which presumably requires metabolic energy.

*Melampus* possesses several physiological mechanisms for dealing with water loss once it has occurred: (1) a remarkably high tolerance to extreme, short-term desiccation (up to 80% of body water lost, a value among the highest reported in the animal kingdom (Adolph, 1943; Schmidt-Nielsen, 1975; Machin, 1976)); (2) tolerance to moderate water loss for at least several days and probably weeks; and (3) rapid rehydration once free water is available. The extremely high tolerance to water loss in *Melampus* is necessitated by its high rate of water loss, which is about 1000 times greater than that found, for example, in desert snails (Schmidt-Nielsen *et al.*, 1972; Machin, 1976). Likewise, rapid rehydration is of clear benefit to *Melampus* adults in the highest tidal zones; dehydrated snails residing here have free water available only during the few minutes of slack tide at the high tides of spring cycles.

The repertoire of physiological "protection levels" of *Melampus* for the range of physical factors in its environment also includes tolerance to freezing. Tolerances to freezing, dehydration, and salinity are all physiologically related (Scarth, 1944; Kanwisher, 1955; Oglesby, 1969; Murphy & Pierce, 1975), since all result in elevated blood and cytoplasmic osmolarities. For example, there occurs a four-fold increase in mg tissue/mg $H_2O$ before death from desiccation in *Melampus* (Table I), and Kanwisher (1955) has shown a four-fold elevation in "salt" concentration in frozen littorinid snails. Kanwisher also reports that up to 76% of the body water in some surviving littorinid snails was in ice, and therefore, these snails endured nearly as much water loss (i.e., water removed from cells and frozen) as seen in the dehydration in *Melampus*.

## Acknowledgements

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# SALINITY AND WATER-TYPE PREFERENCES OF FOUR SPECIES OF POSTLARVAL SHRIMP (*PENAEUS*) FROM WEST MEXICO

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**Abstract:** Preference for low salinity inland water may be an important factor in the immigration of postlarval penaeid shrimp from the sea to their nursery grounds, such as estuaries, bays, and coastal lagoons. In the present work, it was shown that four species from west Mexico *Penaeus vannamei* Boone, *P. stylirostris* Stimpson, *P. californiensis* (Holmes), and *P. brevirostris* Kingsley – all prefer salinities less than that of normal sea water. Older or larger postlarvae preferred lower salinities than early postlarvae. Of the four species, *P. vannamei* has the lowest preference and this species predominates in the coastal lagoon fisheries of the area. Postlarvae of *P. californiensis* and *P. brevirostris* were also shown to prefer lagoon water to sea water. The importance of these preferences is discussed in relation to the normal postlarval migrations.

## Introduction

Salinity affects the distribution of many marine and estuarine animals. Some marine species have a life-cycle a part of which includes an estuarine phase. Pearse & Gunter (1957) noted that it was generally the younger or smaller stages of animals which inhabit low salinities, and although there are some exceptions, this certainly applies to the Penaeidae. Reactions to salinity probably vary with age due to physiological changes within the animal. The extent to which animals react to salinity varies between species. McFarland & Lee (1963), for example, found that juvenile and adult *Penaeus setiferus* were better adapted than *P. aztecus* to tolerate low salinities.

It has been suggested that salinity may be an important factor in the migration of postlarval penaeid shrimp (Keiser & Aldrich, 1976). The horizontal salinity gradients generally found in estuarine areas may help the animals to orientate in order to move toward their nursery grounds. Differences in salinity preference between species, therefore, may explain differences in the extent to which the postlarvae penetrate inland waters.

Similarly, the presence of chemical attractants in inland waters has been shown to be important in the orientation of some aquatic vertebrates. Wisby & Hasler (1954) demonstrated that homing salmon are guided by olfactory cues to their parent

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stream. Creutzberg (1961) found that migrating elvers responded to the presence of the scent of inland waters and, in a simulated tidal cycle, he showed that with increased concentration of "scented" waters, during ebb flow, the elvers sank to the bottom where they orientated upstream toward the source. Odum (1970) suggested that estuarine organisms may use gradients of dissolved organic material as "roadmaps" to orientate themselves to the estuaries. In laboratory experiments, Kristensen (1964) found that several species of young fish and the shrimp *P. aztecus* were attracted to bay water more than to sea water. Both Creutzberg and Kristensen showed that, after passing through a charcoal filter, the inland or bay water lost its attractiveness. What the attractive substances are has not been described, although they are probably organic in nature.

There are four common species of penaeid shrimp on the Pacific coast of Mexico which are commercially important: *P. vannamei* Boone and *P. stylirostris* Stimpson belonging to the subgenus *Litopenaeus* Perez-Farfante, and *P. californiensis* (Holmes) and *P. brevirostris* Kingsley belonging to the subgenus *Farfante penaeus* Burukovskii. All four species occur in the area of study which was centered around the coastal lagoons of Huizache and Caimanero near Mazatlan, Sinaloa. There are commercial fisheries both offshore and in coastal lagoons. *P. californiensis* is the dominant species in the Pacific coastal trawler fishery, while *P. vannamei* and *P. stylirostris* predominate in the coastal lagoon fisheries (Rodríguez de la Cruz & Rosales, 1976).

## Materials and Methods

Keiser & Aldrich (1973) have reviewed the types of apparatus which had been described in the literature for salinity choice experiments. These apparatus fall into two main types: the first consists of multiple choice chambers or levels with sharp salinity discontinuities. The second type of apparatus has continuous salinity gradients which lack discontinuities. In the same paper they described a new gradient apparatus for the study of salinity preference of small benthic and free-swimming animals. A modified version of this apparatus was used in the present study to look at the salinity preferences of the four species of postlarval *Penaeus* and to show whether salinity preference varied between species and with age or size of the postlarvae. The same apparatus was used for the choice experiments between lagoon water and sea water.

Two tanks were constructed of 0.3-mm clear Perspex with dimensions 220 × 10 × 10 cm. A wooden frame supported the tanks, one on top of the other, at an angle of 25° to the horizontal. A 235-cm long fluorescent tube was placed 15 cm behind each tank. Red cellophane was wrapped around each tube to produce red light, which was the only illumination during the experiments. Lines parallel to the floor were marked on the outside of the tanks at 5-cm intervals to provide a means of defining the position of the animals in the tank. The vertical height of the horizontal lines ranged from 0–90 cm above the bottom of the tank.

Salinity gradients, ranging from 0–56‰ were produced in the tanks by slowly adding water of varying salinity. The different salinities were obtained by mixing filtered fresh water with aquarium salt – "Aqua-Marin" (Aquatrol Inc.) or "Instant Ocean" (Aquarium Systems). The salinity gradients were measured before and after each experiment. The methods of gradient preparation, measurement, and introduction of animals were as described by Keiser & Aldrich (1973).

In the experiments to test choice of water type, high salinity water (50 and 40‰), made with aquarium salt, was placed in the first 30 cm of each tank. Water of one of the two stock solutions (lagoon water or sea water) was then slowly siphoned into the next 30 cm, and the remainder of the tank filled with the other water type, the salinity of which was 5‰ less than that in the region below. The difference in salinity prevented the two water types from mixing excessively.

The apparatus was housed in a dark room, but although no temperature control facilities were available in the room, water temperatures varied only slightly during the experiments (normally by no more than 1 °C). The temperatures at which experiments were carried out at different times of the year varied, however, between 26 and 32 °C.

The number of postlarvae at each level in the tanks was counted hourly for 24 h, beginning 1½ to 2 h after introduction. An equal number of shrimp was counted out for introduction into both tanks. Differences in the total number observed were due to counting errors during observations, loss of individuals during introduction, and occasional deaths during the experiment. Dead shrimp were not counted. Deaths were probably due to injury or stress during introduction. The small size of postlarval shrimp and their occasional active swimming were the main reasons for errors in counting.

Postlarvae were collected in surface plankton nets (0.5 m mouth diameter, 475 μm mesh) from coastal waters close to Mazatlan. In the laboratory, postlarvae were separated from other organisms and individually anaesthetized with Eugenol (Cambrian Chemicals, England) to permit identification of species using the characteristics described by Mair (1979). Only one species was used for each experiment and the first 30 postlarvae identified were also measured (total body length) to obtain the mean size for the sample. After recovery from the anaesthetic, the postlarvae were held in aerated water until the experiment on the following day. Some experiments involved preferences of older shrimp and shrimp acclimated to low salinity. For these experiments, identified postlarvae were grown in 30-l glass aquaria and fed daily with newly hatched *Artemia* nauplii. The animals were then counted out on the day of the experiment. All four species were tested for salinity preference, but only *P. californiensis* and *P. brevirostris* were used in the water-type preference experiments.

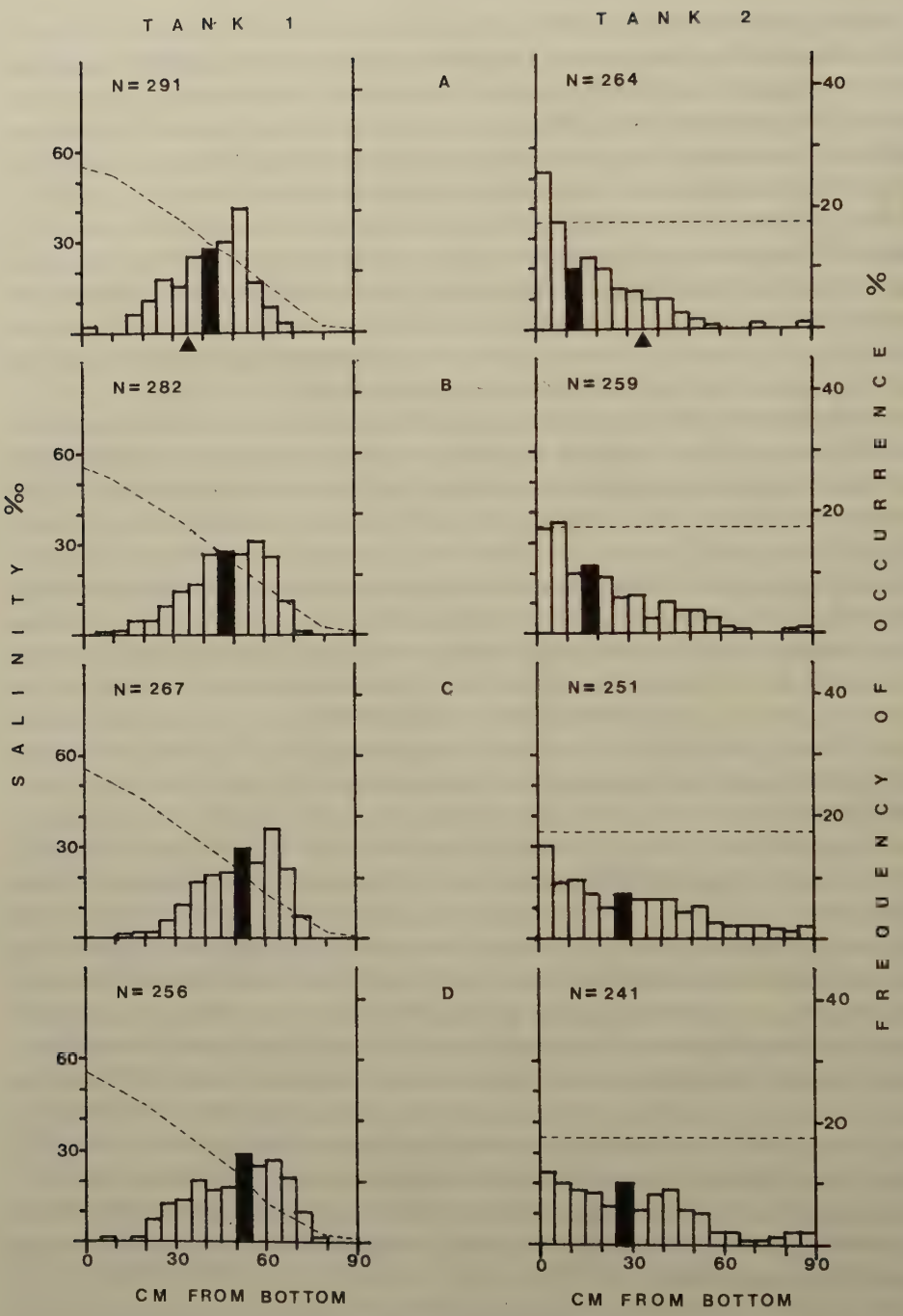


Fig. 1. The distribution of *P. californiensis* postlarvae (9.2 mm mean body length) in a salinity gradient (Tank 1) and in uniform salinity (Tank 2): mean water temperature 26 °C; ▲, level at which the postlarvae were introduced; for other details in this and subsequent figures see text.

## Results

The distributions of animals in experimental and control tanks are shown in Figs. 1–10. The distributions in each of the four 6-h periods (A, B, C and D) are illustrated in Fig. 1, and the dotted lines represent the salinity at each vertical level in the tank. The frequency of occurrence at each 5-cm level over six observations is indicated in the histograms, the black bar representing the position at which the median of the distributions occurred. To study the differences in distribution between tanks and in different experiments, only the final 6-h periods (D) were analysed, using the Kolmogorov–Smirnov two-sample test. To study changes of distribution within an experiment, the initial and final 6-h periods (A and D) were compared.

### SALINITY PREFERENCE

#### P. californiensis

Fig. 1 shows the marked difference in distribution between post larvae of *P. cali-*

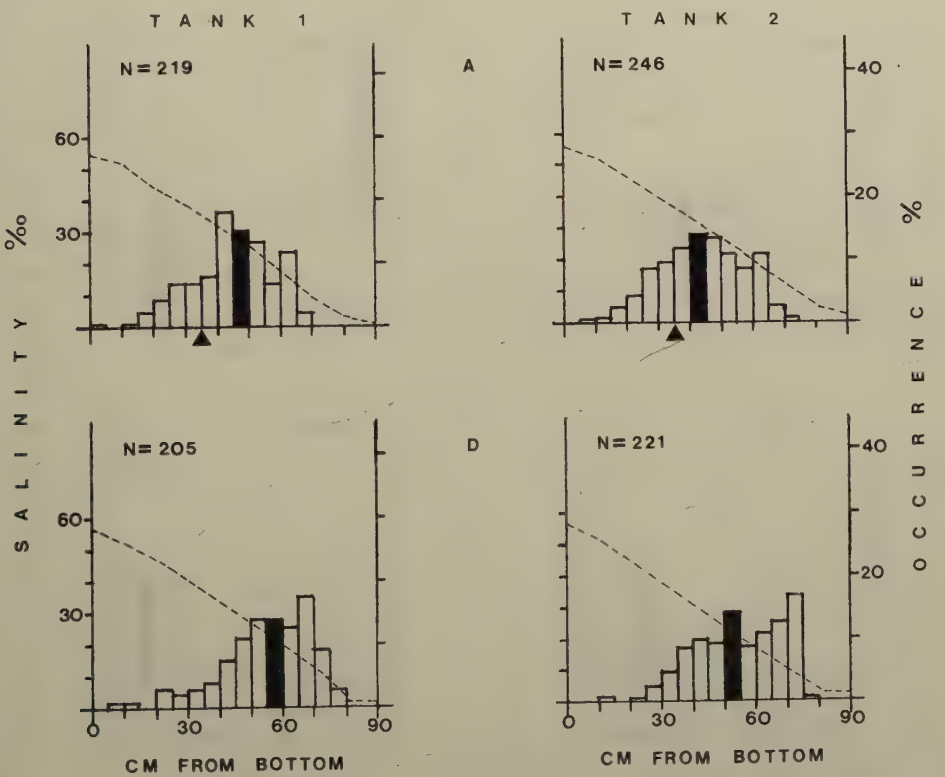


Fig. 2. The distribution of *P. californiensis* postlarvae (9.3 mm mean body length) in two similar salinity gradients in the initial and final 6-h periods (A & D): mean water temperature 26 °C; ▲, level at which the postlarvae were introduced.

*forniensis* in a salinity gradient (Tank 1) and postlarvae in uniform salinity (Tank 2). In uniform salinity, postlarvae occur at all levels, but appear to prefer the lower regions of the tank. In the salinity gradient, extremes of salinity are avoided, and preference is for intermediate salinities. In Period D, the median of the distribution occurs at the 50- to 55-cm level, which corresponds to 19–23‰. In Fig. 2, postlarvae were tested simultaneously in two similar gradients, and there was no significant difference in distribution between tanks in the final 6-h period. In both Figs. 1 and 2, it can be seen that postlarvae move to lower salinities during the experiment, and the distributions are at significantly higher levels (lower salinities) in the tanks during the final period (D) than they were in the initial period (A). Both types of experiment were repeated several times with similar results, although the median values of the distributions in different experiments were often variable.

Fig. 3 shows the distributions of postlarvae, caught on the same day as those used in the experiment illustrated in Fig. 2, but grown in the laboratory for 10 days before

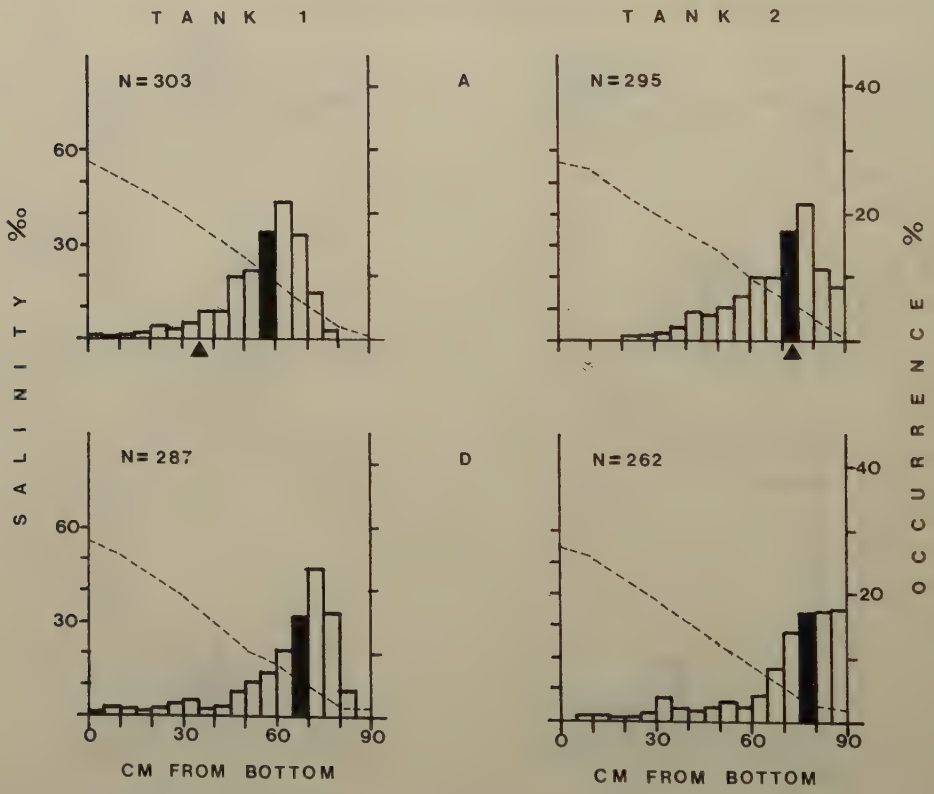


Fig. 3. The distribution of *P. californiensis* postlarvae (12.3 mm mean body length) in two similar gradients in periods A & D: postlarvae were grown for 10 days in the laboratory before testing; postlarvae in Tank 1 were grown in 35‰; postlarvae in Tank 2 were acclimated to 10‰; mean water temperature 26 °C; ▲, level at which the postlarvae were introduced.

being tested. Postlarvae in Tank 1 had been grown in sea water (*S*, 35‰) while those in Tank 2 were grown in water of salinity 10‰. Comparing Figs. 2 and 3, it can be seen that older postlarvae prefer significantly lower salinities than newly caught postlarvae. Acclimation to a salinity of 10‰ results in even lower salinities being preferred. This experiment was repeated with similar results.

P. vannamei

Newly caught *P. vannamei* postlarvae prefer very low salinities (Fig. 4) and this was borne out in duplicate experiments. Tank 2 in Fig. 4 shows the distribution of postlarvae in uniform salinities. Similarly, *P. vannamei* postlarvae grown for 10 days in the laboratory preferred very low salinities.

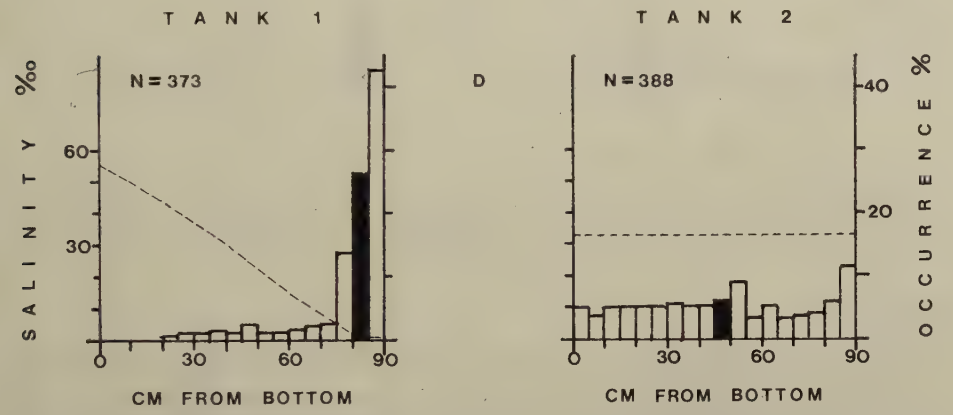


Fig. 4. The distribution of *P. vannamei* postlarvae (5.7 mm mean body length) in a salinity gradient (Tank 1) and in uniform salinity (Tank 2) in the final 6-h period (D): mean water temperature 31 °C.

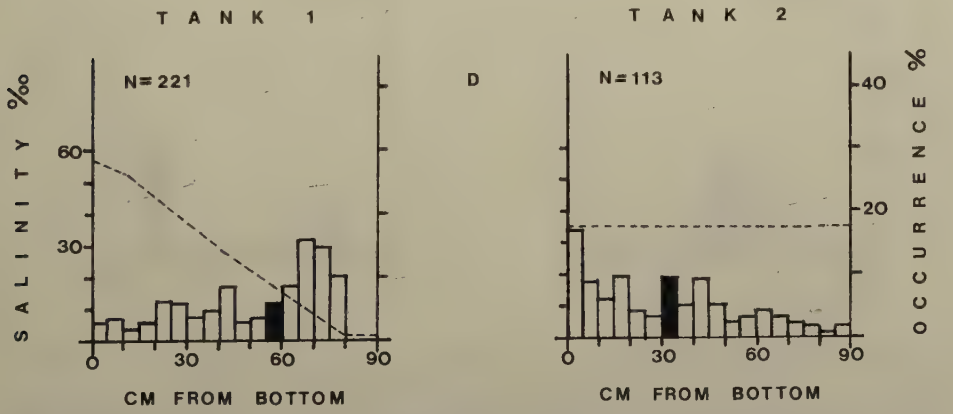


Fig. 5. The distribution of *P. brevirostris* postlarvae (9.4 mm mean body length) for the final 6-h period (D), in a salinity gradient (Tank 1) and in uniform salinity (Tank 2): mean water temperature was 31 °C.

P. brevirostris

Figs. 5 and 6 show the final period distributions of newly caught *P. brevirostris* postlarvae, and those of postlarvae from the same sample but grown for 10 days in the laboratory. The older postlarvae again preferred lower salinities.

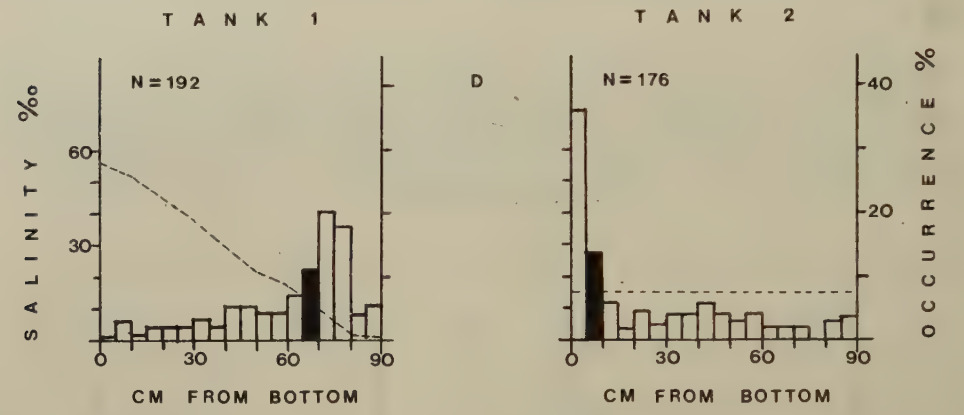


Fig. 6. The distribution of *P. brevirostris* postlarvae (12.5 mm mean body length) for the final 6-h period (D) in a salinity gradient (Tank 1) and in uniform salinity (Tank 2): postlarvae were grown for 10 days at 35‰ before testing; mean water temperature was 29 °C.

P. stylirostris

Newly caught postlarvae of *P. stylirostris* preferred relatively high salinities (Fig. 7) while postlarvae grown in the laboratory for 10 days preferred low salinities (Fig. 8). In this case, however, the postlarvae used in the two experiments were caught

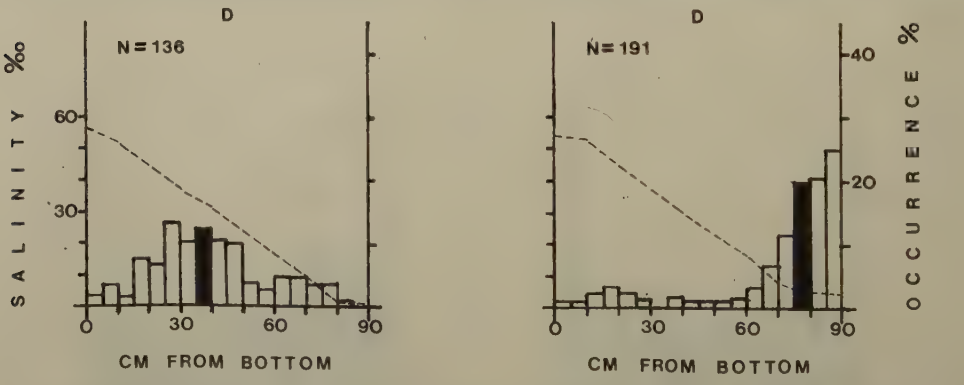


Fig. 7 (left). The distribution of *P. stylirostris* postlarvae (6.0 mm mean body length) for the final 6-h period (D) in a salinity gradient: mean water temperature was 30 °C.

Fig. 8 (right). The distribution of *P. stylirostris* postlarvae (16.9 mm mean body length) for the final 6-h period (D) in a salinity gradient: postlarvae were grown for 10 days at 35‰ before testing; mean water temperature was 30 °C.

on different days. Due to the general lack of abundance of this species, it was not possible to carry out duplicate experiments.

TABLE I

Salinity preference of the four species under various conditions: the median values of the distributions are used to estimate preference ranges; *, results from one experiment only.

| Species | Salinity preference range (‰) | | |
|---|---|---|---|
| | Newly caught postlarvae | After 10 days acclimated to | |
| | | 35‰ | 10‰ |
| *P. vannamei* | 1–8 | 3–6 | 2–3 |
| *P. stylirostris* | 32–35* | 5–7* | – |
| *P. californiensis* | 9–26 | 10–19 | 3–8 |
| *P. brevirostris* | 15–22 | 10–14* | – |

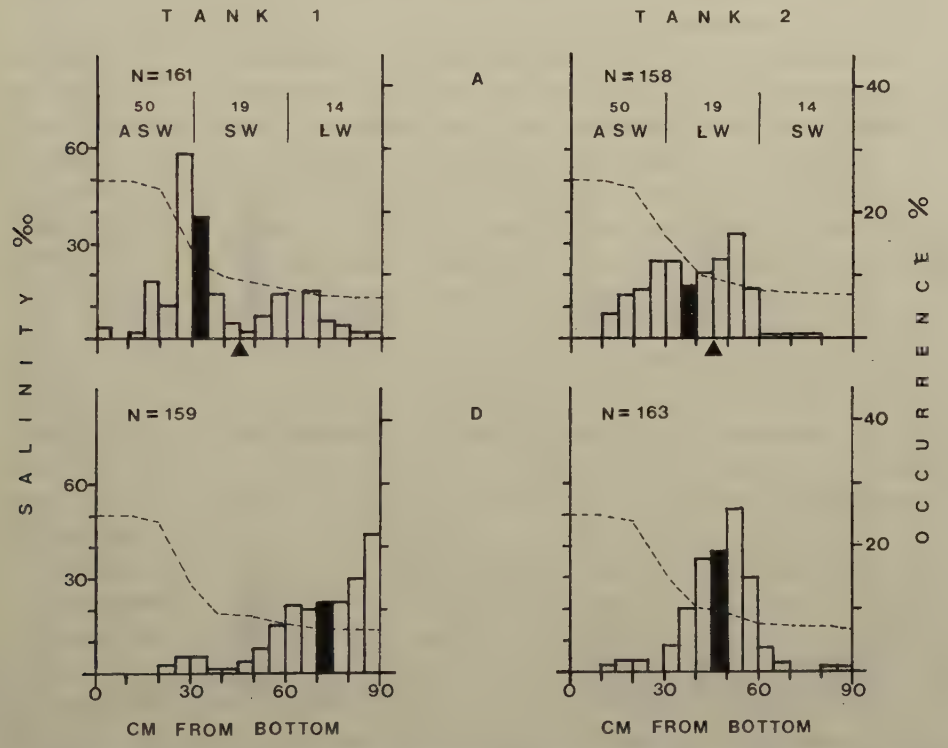


Fig. 9. The distribution of *P. californiensis* postlarvae (7.7 mm mean body length) in gradient tanks containing sections of high salinity artificial water (first 30 cm), and lagoon and sea water: ASW, artificial sea water, SW, sea water, LW, lagoon water; the figues above ASW, SW, and LW indicate the salinity of the water type used; the initial and final 6-h periods (A & D) are shown; mean water temperature 29 °C; ▲, level at which the postlarvae were introduced.

Table I shows the salinity preferences of the four species under various conditions. The salinities occurring in the regions in which the median values fall in the final period of all the experiments are used to give an estimate of salinity preference.

## WATER-TYPE PREFERENCE

When offered artificial sea water, natural sea water, and lagoon water in two different sequences in the gradient apparatus, the distributions in two combinations are clearly very different (Fig. 9). In both tanks, the majority of postlarvae occur in the section containing lagoon water. In Tank 1, however, in the first 6-h period (A), there appears an initial reluctance of postlarvae to move into the upper sections of the tank which contains water of low salinity. This reluctance is overcome in later periods. In three replicates the median values of the distributions were always in the lagoon water section of the tanks, although in a fourth experiment, there was no significant difference between the two distributions.

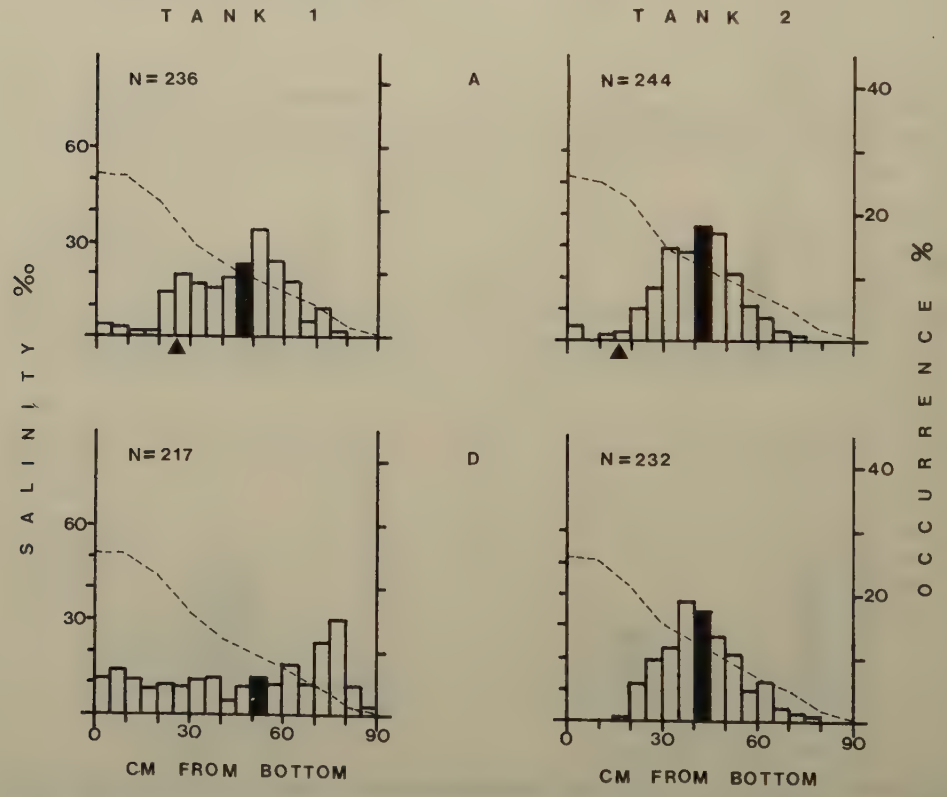


Fig. 10. The distribution of *P. californiensis* postlarvae (10.3 mm mean body length) in two salinity gradients in periods A & D: in Tank 1, the salinity gradient is made with aquarium salt, and in Tank 2 with lagoon water; postlarvae were grown in the laboratory for 7 days at 35‰ before testing; mean water temperature was 29 °C; ▲, level at which the postlarvae were introduced.

In a final experiment (Fig. 10), two salinity gradients were produced: one with aquarium salt water (Tank 1) and the other with lagoon water (Tank 2). *P. californiensis* postlarvae grown for 7 days in the laboratory were tested, and in Tank 1 the preference was for lower salinities than in Tank 2. During the experiment, the distribution shifted towards lower salinities in Tank 1, but in Tank 2, there was no significant difference between the first and final distributions.

## Discussion

### SALINITY PREFERENCE

The distributions of postlarvae in uniform salinities were similar to those found by Keiser & Aldrich (1976) for *P. aztecus* and *P. setiferus* postlarvae. More animals generally occurred at the bottom of the tank, while in other parts they were randomly distributed. It is not clear whether occurrence at the bottom was a response to gravity or barometric pressure, but in any event, it was overridden by the response to low salinity. Accordingly, the bottom 30 cm of the tanks were filled with high salinity water to discourage postlarvae from remaining there. Experiments with salinity gradients showed an avoidance of high salinities and a preference for certain lower salinities in the upper regions of the tanks. In all experiments with *P. californiensis*, the distributions shifted up the tank toward lower salinities.

It must be emphasized that the use of the median value as an estimate of the salinity preference is a qualitative rather than a quantitative measure. Nevertheless, for the majority of the experiments, it gives an indication of the salinity preference of the postlarvae and permits comparison between experiments.

It can be seen from Table I that, of the four species, *P. vannamei* has the lowest salinity preference both in newly caught postlarvae and those grown for ten days in the laboratory. The preferences of *P. californiensis* and *P. brevirostris* are very similar to each other. As explained earlier, the single experiment carried out on newly caught *P. stylirostris* shows them to have a high salinity preference; however, a batch grown in the laboratory for ten days had a very low preference, similar to that of *P. vannamei*.

It appears that salinity preference changes with age or size, as older and larger postlarvae preferred lower salinities. Additionally, the older postlarvae appeared to be able to adapt to lower salinities more quickly. Hughes (1969) tested the reactions of postlarvae to a discontinuity barrier and showed that smaller postlarvae were more averse to moving into a layer of low salinity water than were larger postlarvae. Keiser & Aldrich (1976), however, studying the effects of age and acclimation salinity on salinity preference, found results to be variable in *P. aztecus* and *P. setiferus*.

There was quite a large variation in the distribution of *P. californiensis* postlarvae between experiments. Keiser & Aldrich (1976) demonstrated that the preference of

*P. aztecus* varies with season, attraction towards lower salinities being greatest in the summer. This did not seem to be the case with *P. californiensis* in the present study, and indeed the largest variation between preferences was in two experiments carried out in the same month and under similar experimental conditions.

Throughout all the experiments, the most consistent trend was for larger postlarvae to be attracted toward lower salinities. Although it was not possible to obtain the wholly marine larvae (protozoea and mysis stages) for comparable studies, it seems reasonable to conclude that attraction to increasingly reduced salinities is an important factor in the recruitment of postlarvae to lagoons. Conversely, changes in preference towards higher salinities might be one of the stimuli for the emigration of juvenile shrimp from inland waters to the sea. The observations of Lindner & Anderson (1956) led them to believe that juvenile shrimp move to the sea as a reaction to salinity related to spawning or maturity. Marty-Ordóñez (1972), using a large scale gradient tank, showed that adults of *P. stylirostris* preferred salinities between 35 and 38‰.

Lindroth (1949) introduced the concept of stable and unstable preferences. Species with stable preferences did not seem to be influenced by previous treatment or changes in environmental factors. Lindroth suggested that in eurytopic species, the unstable preference may be the normal one, as appears to be the case with *Penaeus* postlarvae. Jansson (1962) and McLusky (1970) working on oligochaetes and amphipods, respectively, showed, however, examples of eurytopic species having stable preferences.

Rodríguez de la Cruz & Rosales (1976), in reviewing the biology of the four species of shrimp, concluded that *P. vannamei* shows a definite preference for protected waters with low salinities, while *P. californiensis* and *P. brevirostris* are not so dependent on such conditions. The above experiments support these views, at least with respect to the salinity preference of postlarvae.

## WATER-TYPE PREFERENCE

In four out of a set of five experiments, there appeared a marked preference for lagoon water over sea water, and this seemed to override any salinity preference. In the first 6-h period (A) in Fig. 9, however, the salinity drop was so great (from acclimation salinity of 35‰ to 19‰) between the bottom and middle sections that the postlarvae delayed moving up the tank until later periods. It is interesting to note that this only occurred in Tank 1, where the middle section was composed of 19‰ sea water. In Tank 2, with the middle section composed of lagoon water, the postlarvae appeared to have no problem in moving into the low salinities. In this case, the attractiveness of lagoon water overcame the initial avoidance of low salinity.

The comparison of two salinity gradients in Fig. 10 shows the normal shift toward lower salinities in Tank 1, in which the gradient was made from aquarium salt. In Tank 2, however, the distribution did not change throughout the experiment,

and at all times the animals were at lower levels in Tank 2 (lagoon water gradient) than in Tank 1. This suggests that the postlarvae remained in the part of the gradient where the concentration of the attractive substance had not been greatly diluted. It also suggests that a chemical factor other than salinity may be important in affecting postlarval distribution.

Postlarval sampling in the sea indicated that most postlarvae are close to the shore. In this area, they would be more likely to encounter conditions associated with river discharge, such as low salinities and concentrations of dissolved terrigenous material. In the experiments described above, postlarvae showed a preference for both these conditions and, in the sea, they may use horizontal gradients of salinity and varying concentrations of terrigenous factors to orientate towards the river mouths and the associated coastal lagoons. Attraction to the vicinity of the river mouths would increase the chances of postlarvae being carried in on the next high tide when sea water enters the river mouths and moves into the connecting lagoons.

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# THE BIOCHEMICAL COMPOSITION OF PHYTOPLANKTON IN AN UPWELLING REGION OFF SOUTH AFRICA

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**Abstract:** Variations in the concentrations of chlorophyll *a*, ATP, protein, and carbohydrate in phytoplankton have been investigated in a nearshore upwelling region off the Cape Peninsula. During active upwelling temperatures $<10\,°C$, high nutrient concentrations and low concentrations of the biochemical constituents were measured. When upwelling lessened and conditions stabilized temperatures increased and blooms of phytoplankton appeared. High concentrations of chlorophyll *a* and ATP and a high protein/carbohydrate ratio were then recorded. At very low nutrient levels chlorophyll *a* and ATP concentrations were still high but an increase in the acid-soluble carbohydrate content and a corresponding decrease in the protein/carbohydrate ratio was observed. It was concluded that the ratio of protein to carbohydrate was a suitable indicator of the physiological state of a phytoplankton community in the local upwelling region.

## INTRODUCTION

There are rapid chemical and biological changes along the west coast of South Africa during the summer months. Under the influence of southeasterly winds intense upwelling takes place, with high levels of nutrients being brought into the surface euphotic layer. Once conditions stabilize dense blooms of phytoplankton may appear in these waters (Andrews & Hutchings, in press).

In coastal waters off the Cape Peninsula, monitoring of the marine environment has revealed, on the basis of temperature and the concentration of nutrients, three types of water (Table I). For Type 1 water temperatures were $<10\,°C$, while the concentrations of phosphates, nitrates, and silicates were high. These measurements indicated that there was active upwelling of cold water. When upwelling lessened and conditions stabilized temperatures increased to $>10\,°C$ and high or adequate concentrations of nutrients for phytoplankton growth prevailed (Type 2 water). In Type 3 water nutrient concentrations were low with temperatures still $>10\,°C$. These types followed sequentially after water had upwelled and merged with other water bodies.

As the water changed from one type to another variations in the physiological state of the phytoplankton community were expected. To examine these physiological changes appropriate factors needed to be studied in relation to the variations in temperature and nutrient concentration. Chlorophyll *a*, ATP, protein, and carbohydrate are all important biochemical constituents of phytoplankton cells (Stewart, 1974) and were selected as suitable factors for such a study. Chlorophyll *a* is unique

to plant cells whereas ATP, not being associated with non-living material, is only present in living organisms (Holm-Hansen & Booth, 1966). Protein and carbohydrate are metabolites whose concentrations in algal cells have been found to vary in accordance with the physiological state of a phytoplankton community (Antia *et al.*, 1963; Haug *et al.*, 1973).

TABLE I

Water types in the local upwelling ecosystem; Type 1, recently upwelled water; Type 2, maturing upwelled water; Type 3, aged upwelled water.

| | Type 1 | Type 2 | Type 3 |
|---|---|---|---|
| Temperature, °C | <10 | >10 | >10 |
| Nitrate, μg-at. $NO_3$-N/l | >15 | >2 | <2 |
| Silicate, μg-at. $SiO_3$-Si/l | >15 | >2 | – |
| Phosphate, μg-at. $PO_4$-P/l | >1.5 | – | – |

This paper reports the results of a preliminary investigation undertaken during the summer of 1977–78 to establish whether chlorophyll *a*, ATP, protein, and carbohydrate were suitable indicators of physiological change in relation to changes in the environment in the Cape Peninsula upwelling region.

## Methods and Materials

A single station was established at the base of an upwelling plume in 40 m of water, 2 km off Oudekraal (33°58′S : 18°20.6′E). Daily sampling was conducted over 3- to 5-day periods to total 20 days during the months of November, December, and February. This plan of sampling resulted in different bodies of water being monitored as they moved past the Oudekraal station. On each day samples were drawn only from within the euphotic zone at intervals corresponding to 100, 50, 25, 10, and 1% light levels. The 1% light level was taken as the lower limit of the euphotic zone.

The analysis for nutrients was performed on a Technicon Autoanalyser using the methods of Armstrong *et al.* (1967), and Strickland & Parsons (1972) for phosphate and nitrate (including nitrite) and Grasshoff (1965) for silicate. Temperatures were recorded with a bathythermograph and a hand held thermometer.

For the analysis of chlorophyll one l of water was filtered through a Whatman GF/F glass filter, 4.7 cm in diameter, pre-covered with 100 mg of magnesium carbonate. Chlorophyll *a* was determined by the spectrophotometric method of Strickland & Parsons (1972) correcting for phaeopigments. For ATP analysis 0.5 l was filtered through a Whatman GF/F glass-fibre filter and the filters were immediately frozen in liquid nitrogen. Filters were held in liquid nitrogen until analysed. The method of Holm-Hansen & Booth (1966) was used to assay for ATP using a Packard Prias

liquid scintillation counter. Duplicate analyses were made on the extract from each filter.

Plankton samples for protein and carbohydrate analysis were collected by cascading 500–2000 l of water through 200-$\mu$m and 37-$\mu$m plankton nets. Plankton collected on the 37-$\mu$m net was temporarily stored in a refrigerated dark container and transferred to the laboratory within 8 h. The samples were centrifuged, briefly washed, re-centrifuged, frozen, and freeze-dried. The freeze-dried samples were stored at 5 °C until analysed.

Protein was extracted and assayed as described by Mayzaud & Martin (1975) using the Folin–Ciocalteu method of Lowry *et al.* (1951). Bovine serum albumin was used as standard. For total carbohydrate analysis 1 ml of 80% sulphuric acid was added to 1 mg of dry sample (Myklestad & Haug, 1972). After 20 h at 20 °C the mixture was diluted and carbohydrate determined by the phenol-sulphuric acid method of Dubois *et al.* (1956) using glucose as standard.

Carbohydrates were fractionated into acid- and alkali-soluble fractions according to the method of Haug *et al.* (1973). About 10 mg of dry plankton were extracted twice with 0.1 N $H_2SO_4$ (10 ml) for 2 h at room temperature and the extracts were combined. The residue was then extracted twice with 0.1 N NaOH (10 ml) for 2 h and the extracts combined. The phenol-sulphuric acid method, using glucose as standard, was used to determine the amount of carbohydrate in each extract. The amount of insoluble carbohydrate was estimated as the difference between total and soluble carbohydrate. Results for protein and carbohydrate analysis are the means of duplicate analyses expressed as percentage of dry matter.

## Results

During the period of study samples were drawn from 96 various depths. Of this total 30% were drawn from Type 1 water, 51% from Type 2, and 19% from Type 3. Therefore, on most days water containing high or adequate nutrients for phytoplankton growth was monitored at the Oudekraal station.

To investigate possible relationships between the various factors the product moment correlation coefficient ($r$) was calculated between all pairs of measured factors (Tables II and III). The negative correlations between physiological factors

Table II

Correlation coefficients between environmental and physiological factors ($P < 0.01$ except $^*P > 0.05$).

| | Chlorophyll *a* | ATP | Protein | Carbohydrate |
|---|---|---|---|---|
| Temperature | 0.325 | 0.490 | 0.459 | 0.320 |
| Phosphate | −0.440 | −0.556 | −0.512 | −0.423 |
| Nitrate | −0.526 | −0.648 | −0.441 | −0.195* |
| Silicate | −0.579 | −0.668 | −0.337 | −0.089* |

and nutrients were expected. Nutrient concentrations were high when chlorophyll *a* and ATP concentrations were low, but low when the population had increased. Chlorophyll *a* and ATP showed higher correlations with silicate and nitrate than with phosphate, whereas protein and carbohydrate showed a higher correlation with phosphate. A very significant correlation was obtained between chlorophyll *a* and ATP.

TABLE III

Correlation coefficients between physiological factors ($P < 0.01$ except $*P > 0.05$).

| | Chlorophyll *a* | ATP | Protein |
|---|---|---|---|
| ATP | 0.815 | – | – |
| Protein | 0.332 | 0.545 | – |
| Carbohydrate | 0.047* | 0.219* | 0.572 |

Some examples of the results obtained for the three types of water are presented in Figs. 1, 2, and 3. Fig. 1 shows the vertical distribution of measured factors in Type 1 water. The temperatures were all $<10$ °C and the concentrations of nutrients were all very high. Phosphates were between 4 and 6 μg-at. $PO_4$-P/l and nitrates and silicates were in the region of 20 μg-at. $NO_3$-N or $SiO_3$-Si/l. Under these conditions the chlorophyll *a*, ATP, protein, and carbohydrate concentrations were very low.

Type 2 water is illustrated in Fig. 2 where temperatures were between 10 and 11 °C in the upper 10 m. Nutrient concentrations were lower than those recorded for Type 1 water but down to 10 m nitrate and silicate concentrations were still between 2 and 12 μg-at. $NO_3$-N or $SiO_3$-Si/l with phosphates between 1 and 2 μg-at. $PO_4$-P/l. At the bottom of the euphotic zone Type 1 water still prevailed and chlorophyll *a* and ATP concentrations were very low at this depth. In the upper layers of the euphotic zone chlorophyll *a* concentrations were $>10$ μg/l and the ATP concentration $>0.5$ μg/l. On this occasion the highest concentrations of these factors were measured at the surface. It appears that light inhibition had not occurred and nutrients were assimilated faster in the upper layers of the euphotic zone. The protein to carbohydrate ratio was 3.6 at 5 m and between 2 and 3 deeper in the water column.

In Fig. 3, which illustrates Type 3 water, the temperatures are shown to be in the region of 13 °C. Nutrient concentrations were very low with phosphates and nitrates being $<1$ μg-at. $PO_4$-P or $NO_3$-N/l and with no silicate being detected at all. The chlorophyll *a* and ATP concentrations remained high but the carbohydrate content was much greater than the protein content resulting in a ratio of protein to carbohydrate of $<1$.

A comparison between the protein and carbohydrate results for Type 2 and Type 3 water showed that the protein content was similar for the two water types, but the carbohydrate content of Type 3 water was about two to three times higher than that of Type 2 water. Fractional analysis of the carbohydrates for Type 3 water is

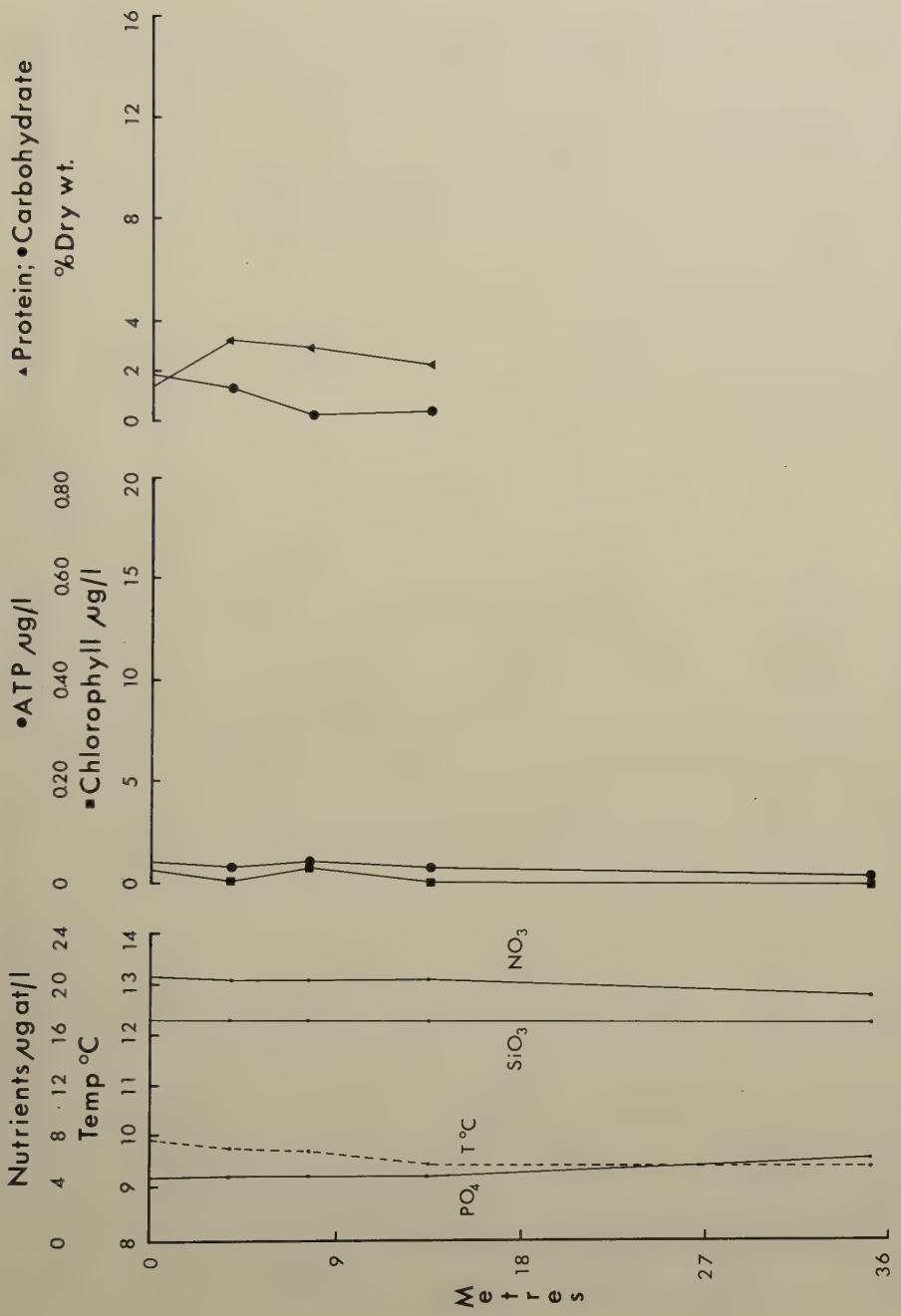


Fig. 1. Vertical distribution of environmental and physiological factors in Type 1 water.

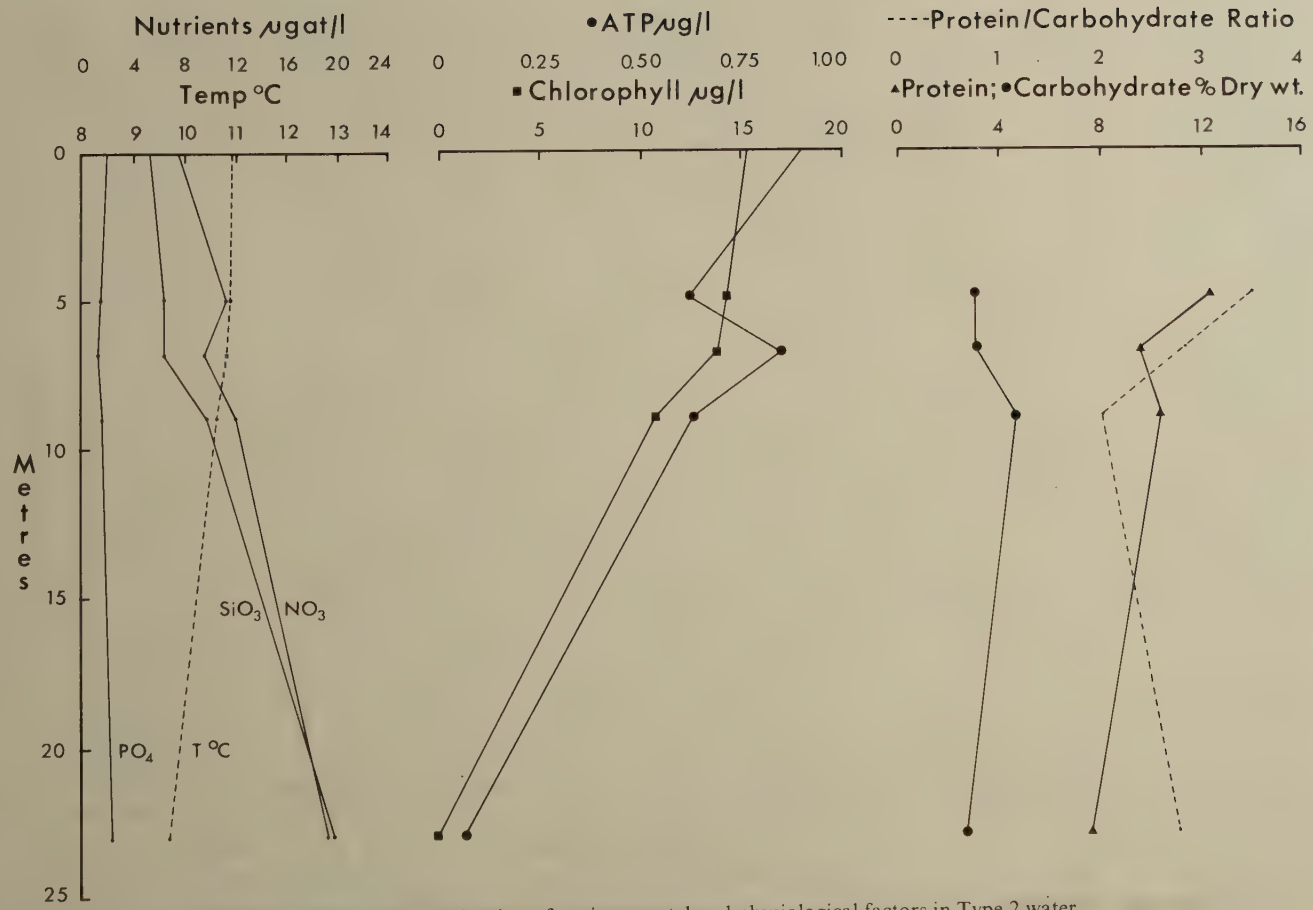


Fig. 2. Vertical distribution of environmental and physiological factors in Type 2 water.

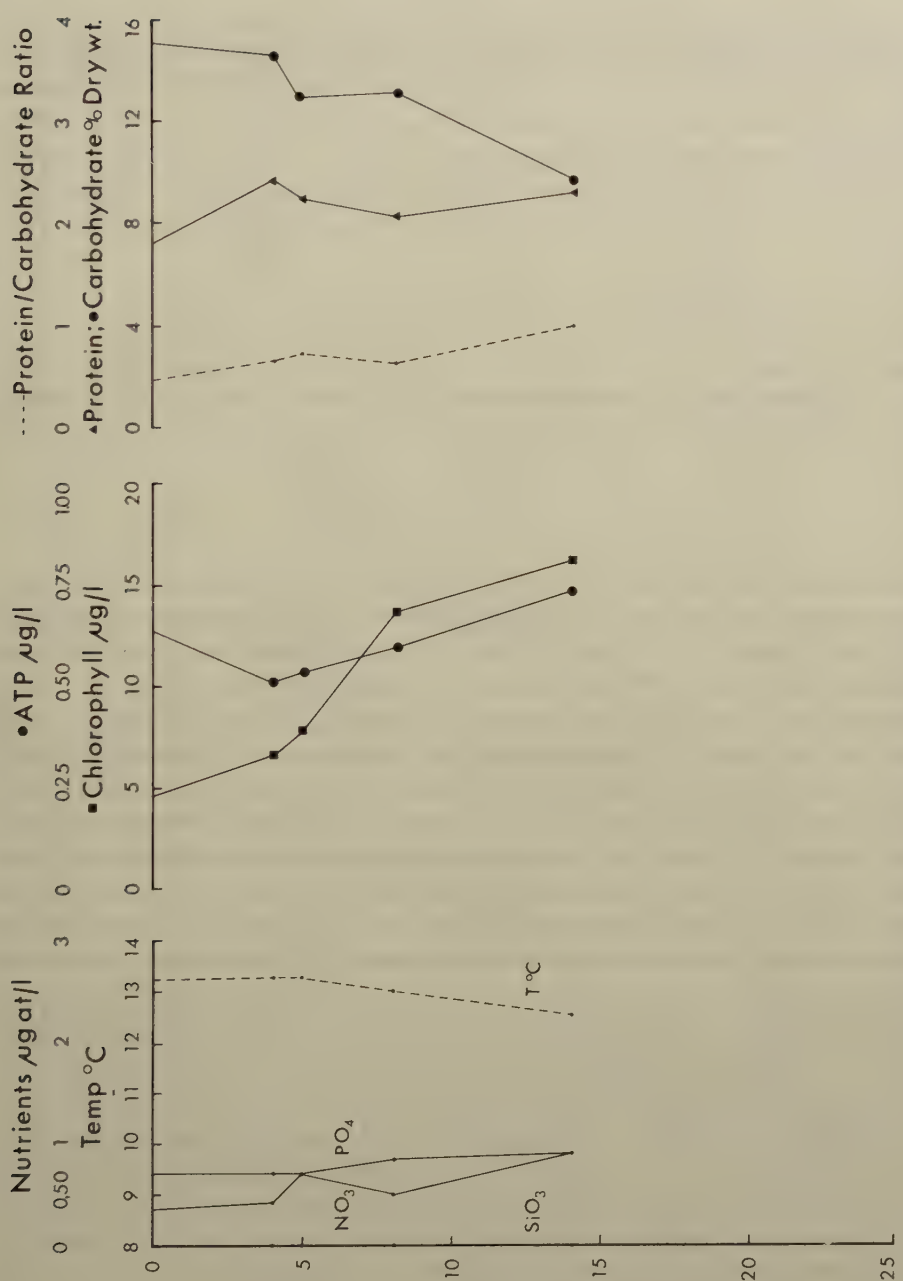


Fig. 3. Vertical distribution of environmental and physiological factors in Type 3 water.

TABLE IV

Carbohydrate composition of phytoplankton as percentage of dry matter for Type 3 water.

| Depth (m) | Total carbohydrate | Acid-soluble fraction | Alkali-soluble fraction | Insoluble fraction |
|---|---|---|---|---|
| 0 | 17.05 | 9.18 | 3.15 | 4.72 |
| 4 | 16.64 | 9.25 | 3.76 | 3.63 |
| 5 | 13.29 | 7.60 | 3.58 | 2.11 |
| 8 | 13.67 | 7.05 | 3.56 | 3.06 |
| 14 | 10.03 | 6.04 | 3.28 | 0.71 |

presented in Table IV and it may be observed that the acid-soluble fraction contributed over 50% to the total carbohydrate concentration. The significance of this will be discussed below.

## DISCUSSION

This investigation has revealed that the changes in the nutrient and chlorophyll *a* concentrations were similar to the results from other upwelling areas (Strickland *et al.*, 1969; Estrada, 1974; Coste & Slawyk, 1974). Correlations between environmental and physiological factors indicated a relation between changes in the environment and changes in the concentration of physiological factors. Figs. 1, 2, and 3 illustrate the nature of these changes. The concentrations of chlorophyll *a* and ATP in Type 3 water were, however, at variance with the results from culture experiments where chlorophyll *a* and ATP were measured (Sakshaug, 1977; Sakshaug & Holm-Hansen, 1977), and field studies on phytoplankton pigments (Jensen & Sakshaug, 1973). These studies indicated that chlorophyll *a* and ATP concentrations decreased at low nutrient concentrations, but high concentrations of these factors were measured by the author in low nutrient waters on several occasions. A possible explanation of the author's findings is that the local phytoplankton populations had not been exposed to the low nutrient conditions for a long enough period for a significant decline in the concentrations of chlorophyll *a* and ATP to occur, even though the protein/carbohydrate ratio indicated a change in nutritional status.

The significant correlation between chlorophyll *a* and ATP compares favourably with the findings of Fiala & Jacques (1974) off North West Africa. Encountering a wide variety of ecological situations, Fiala & Jacques found a significant correlation of 0.638 between chlorophyll *a* and ATP. They concluded that ATP was closely related to phytoplankton except near the limits of the euphotic zone.

The negative correlations between chlorophyll *a* and nitrate and silicate, and between ATP and nitrate and silicate indicated that the phytoplankton communities at Oudekraal were composed predominantly of diatoms. Analysis for phytoplankton species composition, presented in Table V, confirmed that diatoms dominated the

phytoplankton populations in all three types of water. *Thalassiosira aestivalis* Gran and Angst and *Skeletonema costatum* (Grev.) Cleve were the dominant species during November and December 1977.

The carbohydrate constituent was the most sensitive of the physiological factors to respond to a change from Type 2 to Type 3 water. As a result the ratio of protein/carbohydrate varied from a value $>1$, when nutrient availability was adequate, to $<1$ when nutrient concentrations were low.

TABLE V

Species composition and counts for selected days (Oudekraal, 1977).

| Date | Depth (m) | Counts (cells/l) | Dominant species | Percentage of total counts | Water type |
|---|---|---|---|---|---|
| 16 Nov. | 0 | 690 000 | *Thalassiosira aestivalis* | 80.0 | 1 |
| | 7 | 365 000 | at all depths | 74.6 | 1 |
| | 11 | 500 000 | | 78.5 | 1 |
| | 18 | 195 000 | | 85.9 | 1 |
| | 36 | 42 500 | | 76.5 | 1 |
| 17 Nov. | 0 | 6 183 980 | *Thalassiosira aestivalis* | 93.5 | 2 |
| | 5 | 3 471 932 | at all depths | 92.9 | 2 |
| | 7 | 5 543 339 | | 90.4 | 2 |
| | 9 | 2 542 643 | | 91.7 | 2 |
| | 23 | 153 155 | | 96.6 | 2 |
| 7 Dec. | 0 | 817 500 | *Thalassiosira aestivalis* | 68.2 | 3 |
| | | | *Skeletonema costatum* | 18.0 | |
| | 2 | 2 825 000 | *T. aestivalis* | 35.3 | 3 |
| | | | *S. costatum* | 45.4 | |
| | 4.5 | 5 835 000 | *T. aestivalis* | 19.0 | 3 |
| | | | *S. costatum* | 32.9 | |
| | 10 | 1 847 500 | *T. aestivalis* | 46.8 | 3 |
| | | | *S. costatum* | 37.7 | |

Variations in the protein and carbohydrate composition of diatoms due to nutrient depletion have been indicated by other studies. Antia *et al.* (1963) found a marked increase in the carbohydrate content of phytoplankton grown in a large plastic sphere when the amount of available nitrogen was depleted, while the amount of protein as a percentage of algal weight decreased. Myklestad & Haug (1972), working with a diatom culture, showed that the proportion of acid-soluble carbohydrate was relatively low when adequate nutrients were available, but this fraction increased rapidly with the depletion of nitrate from the growth medium. This led to pronounced variations in the ratio of protein to carbohydrate. A study on the natural phytoplankton population in the Trondheimsfjord by Haug *et al.* (1973) produced similar results. The development of a spring bloom was characterized by a rapid decrease in the protein/carbohydrate ratio, with the concentration of nitrate in the surface layers rapidly decreasing during the same period.

The results of this study indicated that the physiological factors selected for study were sensitive to changes in the environment. While the chlorophyll *a* and ATP concentrations were low in Type 1 water and high in Type 2 water a similar significant difference between Type 2 and Type 3 waters was not observed. The ratio of protein to carbohydrate, however, did show significant differences between Type 2 and Type 3 waters. This ratio is, therefore, being used in further investigations of the growth patterns of phytoplankton in the Cape Peninsula upwelling region.

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# SUBSTRATUM SELECTION AND EGG-CAPSULE DEPOSITION IN *NASSARIUS RETICULATUS* (L.)

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**Abstract:** When offered a range of 14 pairs of different substrata consisting of five brown algae, five red algae, three bivalve shells, and sandstone pebbles, spawning *Nassarius reticulatus* (L.) selected only four species of red algae on which to deposit egg capsules. There was some evidence of a greater preference for the red alga *Gigartina stellata* (Stackhouse). When offered a choice between only *Gigartina* and another red weed, *Polyides rotundus* (Hudson), there was a highly significant preference for the former. Some reasons for the selectivity are suggested and discussed.

## INTRODUCTION

*Nassarius reticulatus* (L.) is a prosobranch gastropod commonly found at and below the low water mark of spring tides on sheltered sandy shores in Britain, frequently where freshwater streams run into the sea. It is a euryhaline and eurythermic species (Eriksson & Tallmark, 1974). In the Firth of Clyde, Scotland, *N. reticulatus* breeds in May–July (Allen, 1962) and the eggs are laid within transparent capsules which are a distinctive flattened flask-shape (Ankel, 1929), measuring about 5 mm long and 4 mm wide (Fretter & Graham, 1962). The capsules are not anchored to the sandy substratum in which the adult normally lives but, according to Fretter & Graham, who give an account of their formation and deposition, "... are fastened to any hard object in the vicinity ..." by a broad attachment at the base.

At Millport, Firth of Clyde, it was found that most of the egg capsules collected from the shore were attached to red algae such as *Gigartina* and *Polyides* growing on rocks protruding from the sand near the low water mark of spring tides. Even in the laboratory *Nassarius reticulatus* showed a tendency to lay egg capsules on available red algae although when no weed was available they laid eggs on the sides of the aquarium tanks.

This paper describes the results of experiments to determine the extent of substratum selection by egg-laying *N. reticulatus*.

## Selection from a Range of 14 Pairs of Substrata

### METHODS

In the laboratory, animals were kept on a 2–3 cm deep layer of sand at the bottom of a 100 × 25 × 15 cm deep plastic tank of similar design to that used by Pearce & Thorson (1967) for experiments with another prosobranch, *Neptunea antiqua*. The tank was supplied with running sea water at one end and an overflow at the other.

Two samples each, of 14 examples of various brown and red algae, dead bivalve shells, and stones, commonly found on the shore at Millport, were placed on the sand surface in the tank. The brown algae were *Pelvetia canaliculata* (L.), *Ascophyllum nodosum* (L.), *Fucus vesiculosus* (L.), *F. spiralis* L., and holdfasts of *Laminaria saccharina* (L.). The red algae were *Dumontia incrassata* (O. F. Müll.), *Ahnfeltia plicata* (Hudson), *Gigartina stellata* (Stackhouse), *Chondrus crispus* Stackhouse, and *Polyides rotundus* (Hudson) (nomenclature according to Dixon & Irvine, 1977). It was recognized that *Pelvetia* is never found attached to the shore in the *Nassarius* zone (about M.L.W.S.) since it occurs only in the splash zone of rocky shores. It was included, however, since it has some morphological similarities with the red algae *Polyides* and *Gigartina* and it was thought that it would provide an interesting comparison. The bivalve shells were two single valves each of *Venerupis decussata* (L.), *Dosinia exoleta* (L.), and *Ensis arcuatus* (Jeffreys). The stones were rounded pebbles of sandstone.

All samples of weed, shell, and stone, although of different shapes, were between 4 and 8 cm across, with the exception of the *Ensis* shells which were 12 cm long. The algae were anchored by trapping their stalks in slits cut in small

Table I

Arrangement of weeds, shells, and stones on sand at bottom of experimental tank: the sea-water supply entered the tank at the lower end of the list and the overflow was at the opposite end.

| Row A | Row B |
|---|---|
| 1. *Ahnfeltia* | 7. *Fucus spiralis* |
| 2. *Laminaria* holdfast | 8. *Venerupis* shell |
| 3. *Dosinia* shell | 9. *Polyides* |
| 4. *Gigartina* | 10. *Ascophyllum* |
| 5. *Pelvetia* | 11. Sandstone pebble |
| 6. *Chondrus* | 12. *Fucus vesiculosus* |
| 7. *Fucus spiralis* | 13. *Dumontia* |
| 8. *Venerupis* shell | 14. *Ensis* shell |
| 9. *Polyides* | 1. *Ahnfeltia* |
| 10. *Ascophyllum* | 2. *Laminaria* holdfast |
| 11. Sandstone pebble | 3. *Dosinia* shell |
| 12. *Fucus vesiculosus* | 4. *Gigartina* |
| 13. *Dumontia* | 5. *Pelvetia* |
| 14. *Ensis* shell | 6. *Chondrus* |

rubber bungs which were then buried in the sand, leaving the seaweed exposed above the sand surface. The samples were arranged in two parallel rows along the length of the tank (Table I), each row containing one example of each weed, shell, and stone. No attempt was made to randomize the arrangement, the samples occurring in the same order in each row and the rows being displaced in relation to one another by half a row's length. Within each row, dissimilar samples were positioned next to each other as far as possible.

A sample of 63 *Nassarius reticulatus* was collected from a boulder-strewn sandy beach on the east shore of the Isle of Cumbrae, Firth of Clyde, in June when the animals were in egg-laying condition. The animals were then distributed fairly evenly on the surface of the sand in the bottom of the tank. The experimental tank was examined for egg capsules each day at approximately the same time, when each piece of weed, shell or stone was removed from the tank and all capsules were removed and counted. The same pieces of weed, shell or stone were then replaced in the same positions as before. The experiment lasted for 10 days. Animals were fed with four freshly opened *Mytilus edulis* L. on the second, fourth, and eighth day of the experiment. The temperature varied between 12.5 and 13.8 °C.

## RESULTS

Within 30 min of being placed in the tank the animals were crawling over the sand surface, stones, *Venerupis* shells, *Pelvetia*, and various red algae. After 3 h in the tank most animals had burrowed into the sand but 10 were still crawling over or around the *Polyides* and *Gigartina* plants and appeared to show little or no interest in the other plants, shells or stones. After 24 h the first egg capsules had been deposited. The sex ratio and numbers of females spawning during the experiment were not determined.

TABLE II

Number of egg capsules deposited each day on four species of red algae: no capsules were deposited on any of the other algae, shell or stones listed in Table I.

| Days from start | 1. *Ahnfeltia* | | 4. *Gigartina* | | 6. *Chondrus* | | 9. *Polyides* | |
|---|---|---|---|---|---|---|---|---|
| | A | B | A | B | A | B | A | B |
| 1 | 0 | 0 | 0 | 3 | 0 | 5 | 4 | 1 |
| 2 | 0 | 0 | 0 | 7 | 0 | 1 | 1 | 0 |
| 3 | 0 | 0 | 3 | 1 | 0 | 1 | 0 | 0 |
| 4 | 1 | 1 | 1 | 6 | 0 | 0 | 5 | 3 |
| 5 | 0 | 0 | 15 | 13 | 0 | 6 | 0 | 10 |
| 6 | 1 | 0 | 3 | 3 | 0 | 3 | 3 | 0 |
| 7 | 0 | 0 | 3 | 2 | 0 | 0 | 2 | 0 |
| 8 | 0 | 1 | 0 | 8 | 0 | 4 | 0 | 0 |
| 9 | 0 | 1 | 0 | 4 | 0 | 1 | 0 | 0 |
| 10 | 0 | 0 | 1 | 8 | 0 | 0 | 0 | 0 |
| Total | 2 | 3 | 26 | 55 | 0 | 21 | 15 | 14 |

Table II shows the number of egg capsules removed from plants on each day of the experiment. A total of 136 capsules were laid during the 10-day period. The capsules were laid only on seven plants of four of the five species of red algae and no capsules were found on any of the brown algae, the bivalve shells or the stones. Furthermore, the snails made no attempt to lay capsules on the sides of the experimental tank.

In testing the null hypothesis that all the objects were equally attractive, the probability of the snails choosing only 7 objects in 4 pairs out of 28 objects in 14 pairs can be calculated as $2\binom{4}{1}\binom{14}{4}/\binom{28}{7} = 0.0068$. Given that 7 objects were selected, the event that they belong to only 4 species is the most extreme possible event demonstrating selection of weed species. Thus, the choice of 7 out of 8 plants belonging to 4 species of red algae was highly significant.

When the stones and shells are ignored and only the 10 species (20 plants) of seaweed considered as equally attractive the probability of the snails selecting only 7 red algae from 4 species is $2\binom{4}{1}\binom{10}{4}/\binom{20}{7} = 0.022$ and is still significant.

The number of capsules found on each species of weed (Table II) suggests that the animals may have shown preferences for certain species of the red algae. *Gigartina* received 81 capsules, *Polyides* 29, *Chondrus* 21, and *Ahnfeltia* only 5 whilst no capsules were laid on the remaining red alga, *Dumontia*.

The differences between numbers of capsules laid during the experiment on any two species of weed, or on any two plants of each species, were tested with the Wilcoxon signed ranks test using the differences between the daily matched pairs (Conover, 1971). One of the assumptions of the test is that the differences for each pair must be independent. It might be argued, therefore, that basing each test on successive daily differences between the same pair is invalid. The fact that a snail laid capsules on a certain weed one day might enhance the chances of it choosing the same plant on the next and successive days, either because it remained on the plant or returned to it along slime trails. Each weed was, however, removed from the sand for a few minutes every day for examination and removal of capsules, when any snails on the weed dropped back onto the sand. Furthermore, there was no evidence that the snails left slime trails and if they did, daily removal of the weeds would break the continuity of such trails. For these reasons, independence of the differences was assumed for the tests.

The results of the tests for preferences between plants of the four selected species of red weed were rather inconclusive. For example, *Gigartina* plant B was significantly more attractive ($P < 0.05$) than any of the six plants of the other three species whilst there was insufficient evidence of plant A being more attractive than any of the six except *Chondrus* plant A (which received no capsules) and *Ahnfeltia* plant A. Indeed, each of these two plants was consistently less attractive than any of the other species, whilst *Chondrus* plant B and *Ahnfeltia* plant B were not shown to be different from the other species except *Gigartina* plant A.

There were considerable differences between the numbers of capsules on the two

plants of *Gigartina*, with totals of 55 and 26 capsules, respectively. The differences were, however, not significant although the decision to accept the null hypothesis of no differences between the plants was marginal ($P = 0.05$). There was no difference between the two *Polyides* plants ($P = 0.20$) and so for each of these two species the daily capsule counts on the two plants were summed. The numbers of capsules laid on *Gigartina* plants were then significantly larger than *Polyides* ($P < 0.005$). Similar comparisons showed that there were significant preferences for *Gigartina* compared with *Chondrus* and *Ahnfeltia*. Similar comparisons between *Polyides*, *Chondrus*, and *Ahnfeltia*, however, gave only one significant difference with greater preference for *Polyides* than *Ahnfeltia*.

Since there was some evidence that *Gigartina* was chosen more than the other weeds and because *Gigartina* and *Polyides* were two of the commonest weeds in the *Nassarius* habitats of the Clyde (pers. obs.), the second experiment was carried out using only these two weeds to test whether a real difference existed.

## THE CHOICE BETWEEN *GIGARTINA* AND *POLYIDES*

### METHODS

The experiment was a continuation of the previous one, using the same 63 *Nassarius*, which by now had been acclimatized to laboratory conditions for 10 days. All shells, stones, and weeds were, however, removed from the tank and replaced with two freshly gathered plants each of *Gigartina* and *Polyides*. The four plants were arranged so that they marked the corners of a square of side 10 cm in the middle of the tank's floor, with the two plants of one species on diagonally opposite corners. At noon each day the weeds were removed from the tank, examined for capsules and then replaced. The animals were fed with freshly opened *Mytilus edulis* before the start of the experiment and on the 10th and 19th day. The experiment lasted for 25 days. The temperature varied between 11.4 and 14.8 °C.

### RESULTS

A total of 263 egg capsules were laid, always on the four weeds (Table III). 211 capsules (80%) were laid on the two *Gigartina* plants compared with only 52 on the two *Polyides* (20%).

Wilcoxon signed ranks tests on the daily differences in egg capsule counts showed that there were no significant differences between the two plants of each of the two species ($P > 0.05$). The differences between the two species, however, were highly significant with each plant of *Gigartina* receiving significantly more capsules than either of the *Polyides* plants ($P < 0.005$ in three of the four combinations and $P < 0.05$ for plants A of *Gigartina* and *Polyides*).

When offered a choice of only *Gigartina* and *Polyides* the snails conclusively showed a preference for *Gigartina*.

TABLE III

Numbers of capsules laid on two species of red algae in the second experiment.

| Days from start | *Gigartina* | | *Polyides* | |
|---|---|---|---|---|
| | A | B | A | B |
| 1 | 1 | 6 | 4 | 0 |
| 2 | 8 | 6 | 7 | 1 |
| 3 | 2 | 1 | 0 | 0 |
| 4 | 2 | 0 | 0 | 0 |
| 5 | 2 | 3 | 5 | 2 |
| 6 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 |
| 9 | 6 | 3 | 0 | 0 |
| 10 | 12 | 24 | 0 | 2 |
| 11 | 7 | 15 | 0 | 1 |
| 12 | 0 | 5 | 0 | 2 |
| 13 | 9 | 11 | 2 | 6 |
| 14 | 1 | 9 | 1 | 0 |
| 15 | 1 | 2 | 0 | 5 |
| 16 | 0 | 0 | 0 | 0 |
| 17 | 0 | 0 | 1 | 0 |
| 18 | 0 | 2 | 5 | 1 |
| 19 | 9 | 3 | 0 | 4 |
| 20 | 0 | 0 | 0 | 0 |
| 21 | 0 | 0 | 0 | 0 |
| 22 | 0 | 0 | 0 | 0 |
| 23 | 18 | 5 | 1 | 1 |
| 24 | 11 | 10 | 1 | 0 |
| 25 | 9 | 8 | 0 | 0 |
| Total | 98 | 113 | 27 | 25 |

## DISCUSSION

If a sandy shore-dwelling gastropod did not attach its egg capsules to a relatively stable substratum, there would be a risk of the egg capsules being carried up the shore by wave action, where the larvae might die of prolonged exposure. The opportunities of finding stable substrata, however, can be rather limited on the types of sandy sediments normally inhabited by *Nassarius*. Prosobranchs like various species of *Natica* deposit their eggs in the interstices of sand grains formed into characteristic circular egg collars (Fretter & Graham, 1962). Although these are not anchored to the sand surface, their flattened shape reduces the chances of water movements and wave action carrying them away. Some prosobranchs, e.g., *Bullia melanoides*, on tropical sand beaches produce egg capsules which remain attached to the female, who guards them until they are released (Ansell & Trevallion, 1970). Most sand-

dwelling prosobranchs, however, appear to attach capsules to rocks and weeds occurring at intervals on the sand (Fretter & Graham, 1962).

Fretter & Graham also give evidence that egg capsules of *Nassarius reticulatus* are attached to any hard object lying on the sand surface, such as rocks and stones. The results of the present experiments suggest, however, that given a choice of objects, *N. reticulatus* may be considerably more specific.

The first experiment clearly showed that *N. reticulatus* had a marked preference for depositing egg capsules on certain seaweeds instead of on shells and stones. Indeed no capsules were deposited on any of the shells and stones provided. Furthermore, given a variety of 10 different seaweeds, the snails showed a distinct preference for only a small number of species. In the second experiment, it was shown that there was even a highly significant preference for one species of the two similar red algae provided.

Since the basal foot of each capsule is fastened to the substratum by the snail, apparently by adhesion (Fretter & Graham, 1962), it might be assumed that this would be accomplished more easily on rocks and shells, which have a much harder surface than seaweeds. There may, however, be a number of advantages in attaching capsules to weeds rather than stones. Since capsules are small, being only 5 × 4 mm in size, they will be well-camouflaged by the seaweed fronds and less liable to predation by animals (e.g. *Psammechinus* and *Echinus*, pers. obs.) feeding on rock encrusting organisms. The seaweeds also tend to be on larger, more stable stones and rocks which are not likely to be moved by wave action. The weed fronds would also provide considerable protection for the capsules from wave action and turbulence, whilst during exposure in spring tide periods the capsules would receive greater protection from desiccation and high air temperatures.

Stones and small rocks on sandy shores are also liable to be covered by sand during storms and any attached capsules might be smothered. This is less likely to occur with capsules attached to weed.

During the present work, snails kept in laboratory tanks without weed or stones would frequently deposit capsules on the walls of the tanks, although it is not known whether the absence of weed or stones inhibited capsule deposition. On a sandy beach in Ayrshire, containing large numbers of *Nassarius reticulatus* but very few rocks, stones, dead bivalve shells, weeds or any other likely substrata for capsules, the authors have observed very large concentrations of snails, during the breeding season, buried in the sand surrounding the few isolated boulders present. Capsules were found attached to these boulders. On the same beach, A. D. Ansell (pers. comm.) has observed *N. reticulatus* capsules deposited on the unattached sandy egg collars of the prosobranch *Natica* spp. lying on the sand surface. Fretter & Graham (1962) record a similar occurrence. On other sandy beaches in the Firth of Clyde, containing large numbers of rocks with brown and red algae, the authors have collected large numbers of capsules from above and below the low water spring tide level by diving during high tide. On these beaches the capsules were nearly always

fastened to red algae attached to the rocks, and on only a very few occasions were capsules found on rocks or brown algae (the holdfasts of *Laminaria* spp.).

Field observations therefore support the experimental evidence that snails prefer to deposit capsules on algae, rather than rocks, but that in the absence of weed, or when weed is very scarce, capsules will be deposited on rocks and other available hard objects, as described by Fretter & Graham (1962).

The experiments show that, given a choice of different seaweeds, *Nassarius reticulatus* has a significant preference for some species of red algae. The reason may be that it is easier to attach capsules to seaweeds with certain types of surfaces. Most of the brown algae, which were not used by snails for capsule deposition in the experiments, have frond surfaces covered by the mucilages which are a prominent feature of the Phaeophyceae (Boney, 1966). Clearly, it would be difficult for capsules to adhere to such surfaces, although Fretter & Graham (1962) mention that *N. reticulatus* will use the radula to clean the substratum surface before depositing a capsule. On occasions when egg capsules were observed by the authors on *Laminaria* holdfasts in the field, the holdfasts were invariably fairly old with little or no surface mucilage. On the other hand, some of the red algae have only a thin layer of surface mucilage which appears to be virtually absent in the older parts of the fronds. This condition probably makes such weeds attractive to egg-laying *Nassarius*. *Gigartina* has a frond surface which is leathery (Boney, 1966) and this may be one reason why more egg capsules were laid on this species than on the other red weeds. In addition, the shape of the fronds may be important because both *Gigartina* and *Polyides* have narrow stipes and fronds around which the basal anchoring part of each capsule was observed to be partly wrapped by the snails. The greater preference for *Gigartina* may have been caused partly by the grooved stipes of *Gigartina* providing a better attachment for capsules than the round-sectioned stipes of *Polyides*.

Suitable shape alone is probably not always satisfactory since two pieces of the brown alga *Pelvetia canaliculata* were offered in the first experiment and had a frond and stipe form very similar to that of the red weed *Polyides*. Despite the similarity, no capsules were laid on the *Pelvetia*, presumably because of the mucilage coating the surface, whilst capsules were laid on the similarly shaped *Polyides*. The presence of a non-slippery weed surface alone is probably not entirely satisfactory either. The red alga *Ahnfeltia* has virtually no mucilage coating but the fronds are finely divided and thread-like and clearly would provide a very poor shape for the attachment of capsules (Table II). Similarly, the presence in *Dumontia* of a tubular, membranaceous thallus (Newton, 1931) would provide a poor substratum, accounting for the total absence of capsules on this weed.

The attachment of a total of 21 capsules to *Chondrus* plant B but none to plant A in the first experiment is difficult to explain. Like *Gigartina*, *Chondrus* has a leathery thallus (Boney, 1966) and should provide a good substratum, as it did in plant B.

The absence of capsules on plant A might be a chance occurrence but this seems unlikely over a period of 10 days.

In general, however, it would appear that the combination of a non-slippery surface and reasonably narrow stipes and fronds of firm consistency, but not too filamentous, provides the best type of weed substratum for capsule attachment. Both these conditions occur to different extents in the red seaweeds *Gigartina* and *Polyides*. These weeds were very abundant on most of the beaches with *Nassarius reticulatus* populations examined in the Clyde, where collections indicated that most capsules occurred on these weeds.

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# SEASONAL CHANGES IN TISSUE WEIGHT AND BIOCHEMICAL COMPOSITION FOR THE BIVALVE *DONAX TRUNCULUS* L. ON THE ALGERIAN COAST

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**Abstract:** Seasonal changes in the body weight and composition of the bivalve *Donax trunculus* L. from Azur Plage near Algiers, Algeria, were studied during the period September 1971–June 1974. Percentage dry tissue weight was minimal in October December and rose to reach a peak in spring or summer, the rise corresponding with rapid maturation of the gonads. Decrease in tissue weight in the autumn also coincided with a rapid decline in maturity of the gonads between August and October. Lipid content increased with sexual maturity, while carbohydrate content was greatest in the autumn and declined to a seasonal minimum in April–May. The changes are compared with those for *D. trunculus* in other Mediterranean populations, and for *D. vittatus* (da Costa) elsewhere in Europe.

## INTRODUCTION

During a general study of the biology of *Donax trunculus* on the Algerian coast (Moueza, 1975) observations were made on the population structure and seasonal reproductive cycle of a population on the beach of Azur Plage near Algiers. During the course of these observations a series of samples of adult *D. trunculus* from this population were collected for study of seasonal changes in tissue weight and some of these samples were freeze-dried and retained for further biochemical studies. These samples were later analysed at the Dunstaffnage Marine Laboratory and this paper is based on the results of these analyses, together with the original observations on the Azur Plage population. Although there is now an extensive literature on the seasonal cycles in tissue weight and biochemical composition associated with growth and reproduction in northern-boreal bivalves (see Beukema & De Bruin, 1977) information on Mediterranean species is more limited, and it is of interest therefore, to record the changes found in this characteristic beach species for a North African locality.

## Materials and Methods

The *D. trunculus* L. used in this study were collected from the beach at Azur Plage, Algeria, between September, 1971 and June 1974. The physical conditions of the area were described by Degiovanni & Moueza (1972) and the general features of the *D. trunculus* population by Moueza (1972) and Moueza & Chessel (1976). Moueza & Frenkiel-Renault (1973) described the features of the reproductive cycle and of settlement, and Ando *et al.* (1976) variations in lipids and sterols during autumn and winter for the same population.

In the present work, observations were restricted to adult animals (shell length >16 mm). The animals collected were divided into narrow size groups by shell length, and the total fresh weight, shell weight, and wet and dry tissue weights were determined collectively for groups of animals in each size class. These data have been used to examine the relationships between shell length and total weight, and shell length and shell weight, and to calculate wet and dry tissue weights for each sampling date as a percentage of the total fresh weight. Since there were no significant differences over the size range examined in % wet and % dry tissue weights between different size groups, the individual determinations for all size groups for each sampling date have been pooled to give a mean percentage for each sampling date, in examining the seasonal cycle in tissue weight. In addition, for comparison with other published studies (Beukema & De Bruin, 1977; Bodoy & Massé, 1979; Ansell & Bodoy, 1979), a condition index for each sampling date was calculated as dry tissue weight/shell length$^3$, and again since there were no significant differences between size groups, a mean value for each sampling date has been calculated.

Between September 1972 and November 1973, samples of *D. trunculus*, again sorted into narrow size groups, were freeze-dried. These freeze-dried tissues were stored over desiccant and later (1977) used for the determination of carbohydrate, lipid, carbon and nitrogen, and ash by standard methods employed in previous studies at the Dunstaffnage Marine Laboratory (Ansell, 1974a).

In presenting the results, in addition to the figures for percentage composition, the tissue weight and composition may be calculated for an individual of 2.5 g total weight, hereafter referred to as a standard animal. This size of standard animal was chosen for comparison with a previous study of seasonal changes in the northern-boreal species, *D. vittatus* (Ansell, 1972). For *D. trunculus*, from Azur Plage, a standard animal of 2.5 g total weight would have a shell length of 25.6 mm.

## Results

### Length–weight relationships

Regressions of shell length on total fresh weight, and shell length on shell weight were calculated from the pooled sample weights following logarithmic conversion.

The relationships found were as follows:

$$\log \text{shell weight (mg)} = 2.719 \log \text{length (mm)} - 0.627$$

and,

$$\log \text{total weight (mg)} = 2.667 \log \text{length (mm)} - 0.358.$$

The relationship between shell weight and total fresh weight changes slightly with increase in size over the size range examined (16–28 mm shell length) giving the relationship:

$$\text{shell weight/total weight} = 0.052 \log \text{length (mm)} - 0.268.$$

In *D. trunculus,* the two shell valves are unequal, the left valve overlapping the right, e.g., at the dorsal border when viewed anteriorly (Bucquoy *et al.*, 1887–1898). This important diagnostic character for *D. trunculus* is reflected in a difference in weight between the two valves, but the right valve – the smaller one – is consistently heavier in all size classes (Fig. 1).

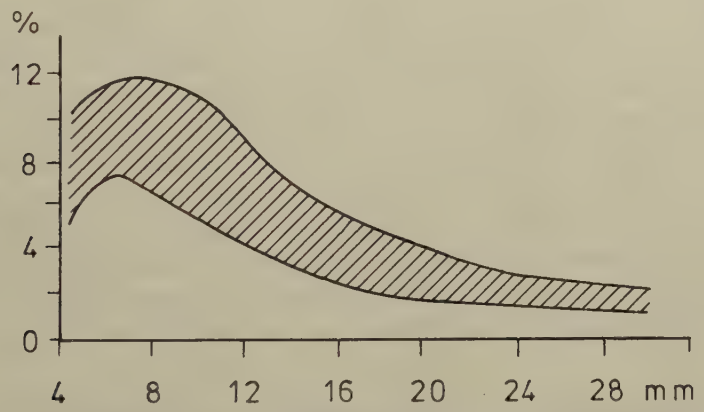


Fig. 1. *Donax trunculus*: differences in weight between the left and right valves of the shell as a function of shell length shown as a percentage of the total weight of the shell.

## SEASONAL CHANGES IN PERCENTAGE TISSUE WEIGHT AND TISSUE CONTENT OF A STANDARD ANIMAL

The changes in wet and dry tissue weight, expressed as a percentage of total weight, for the period October 1971 to June 1974 are summarized in Fig. 2. Since the shell length–total fresh weight relationship does not change, these also represent the tissue content of a standard animal. In each year, the percentage dry tissue was at its minimum in October, November or December and thereafter rose, at first slowly, to reach a peak in the spring or summer. In 1972, a maximum value in April was

followed by a decline although with some recovery in July; in 1973, the early increase was slower and more prolonged and the seasonal maximum was not reached until August.

The percentage wet tissue weight shows a less consistent pattern. Again minimum values were during the autumn but with some variation in the timing between years. This was followed by a somewhat irregular increase to a second maximum in April (1972 and 1974) or June (1973).

The relationship between wet and dry weights, expressed as the water content of the tissues, shows a more consistent pattern with high values during the autumn

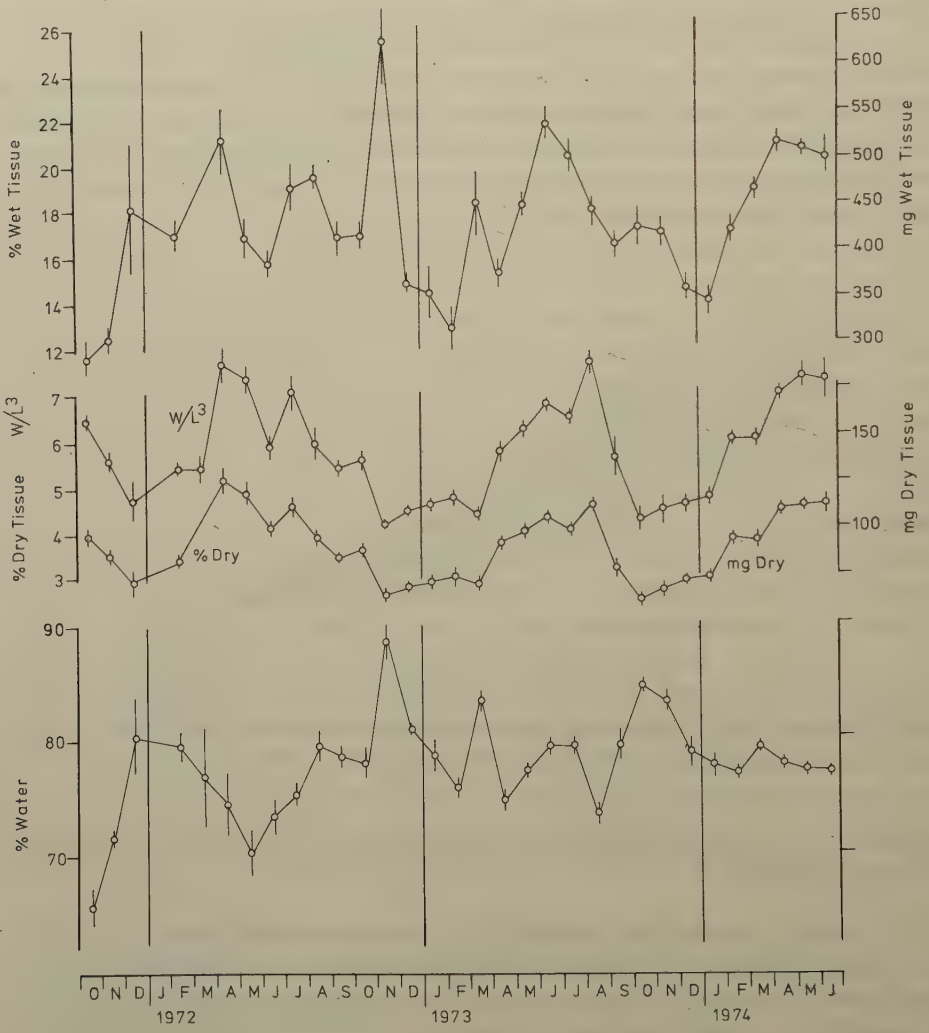
% Wet Tissue
mg Wet Tissue
% Dry Tissue
W/L3
% Dry
mg Dry
mg Dry Tissue
% Water
O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J
1972
1973
1974

Fig. 2. *Donax trunculus*: values for % wet tissue, % dry tissue (together with equivalent values of the wet and dry tissue content of a standard animal of 2.5 g total weight); % water content of the tissues, and of the dry weight condition index ($W/L^3$), from the population at Azur Plage, Algeria.

when tissue content is low. The increase in tissue content in the spring is accompanied by a decrease in water content of the tissues, while thereafter increases in water content reflect first spawning activity in the summer and then the cessation of reproductive activity in the autumn.

## SEASONAL CHANGES IN CONDITION INDEX

The seasonal changes in the dry weight condition index ($W/L^3$) are also shown in Fig. 2. The changes, as would be expected, reproduce closely the cycle in percentage dry tissue weight, with values varying from a seasonal minimum in the winter of $\approx$4.3 to a maximum of $\approx$7.8.

## RELATIONSHIP OF SEASONAL TISSUE WEIGHT CHANGES TO THE REPRODUCTIVE CYCLE

Moueza & Frenkiel-Renault (1973) described the general features of the reproductive cycle for *D. trunculus* at Azur Plage. Between October and January the gonad is not developed and there is an inactive period in the reproductive cycle. From February there is a rapid increase in the number of individuals with ripe gonads, and in the population as a whole this increase continues until August when there is a sudden fall in the numbers of ripe animals, marking the end of the reproductive period. When only the larger individuals used in the present study are considered, this cycle is slightly modified (Fig. 3). Between April and August all the large animals are sexually mature and most are in the fully ripe condition. There is a rapid maturation of the gonads in these animals between January and April, with only minor variations in timing from year to year, and a similarly rapid decline in maturity and ripeness between August and October. These two periods closely correspond with periods of maximum increase in dry tissue weight in the spring, and of decrease in tissue weight between August and October, the latter decrease being

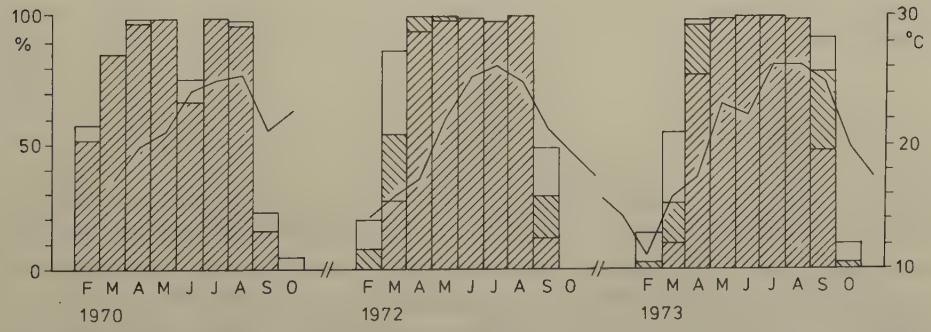


Fig. 3. *Donax trunculus*: the seasonal cycle of gonad development for individuals 16 mm shell length, from the population at Azur Plage, Algeria; sea-water temperature is also given; the shading indicates different stages of gonadal deveopment as defined by Moueza & Frenkiel-Renault (1973).

accompanied by a great uptake of water into the tissues as the gonad regresses leading to seasonal maximum values of water content.

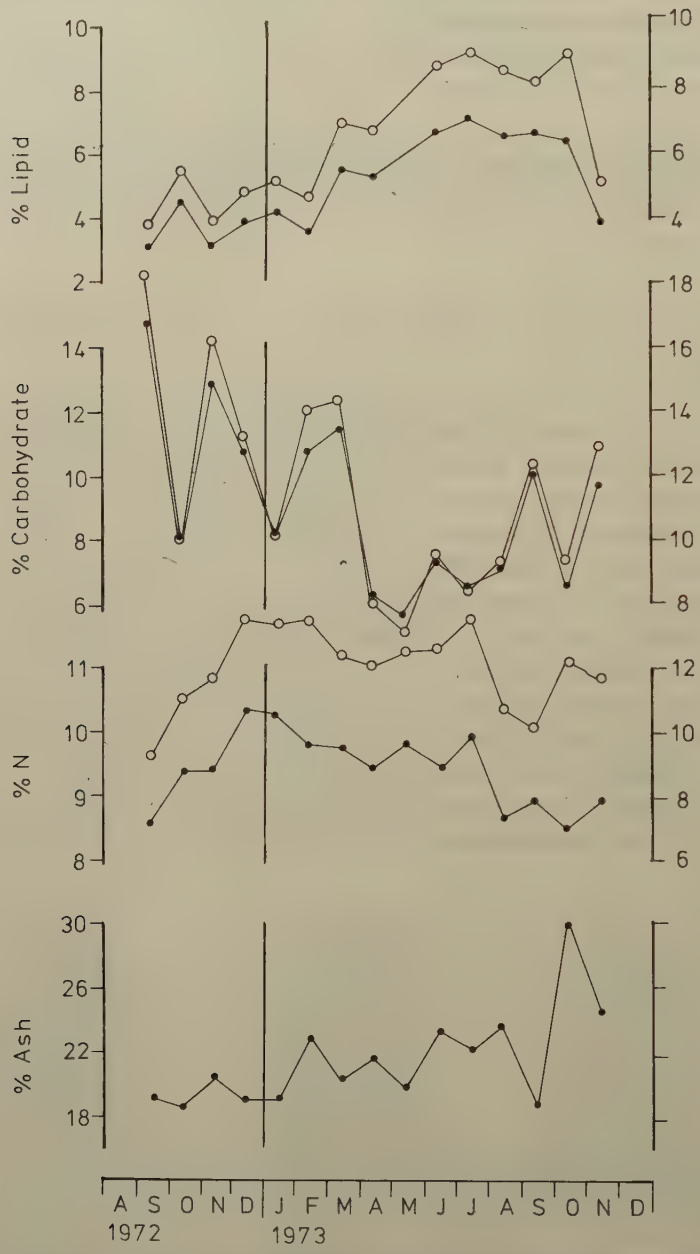


Fig. 4. *Donax trunculus*: changes in % biochemical composition of the population at Azur Plage, Algeria; values for lipids, carbohydrate and nitrogen are shown on both dry weight (●) and ash-free dry weight basis (○).

## SEASONAL CHANGES IN BIOCHEMICAL COMPOSITION

The changes in percentage composition of the dried tissues between October 1972 and November 1973 are shown in Fig. 4. The change in percentage lipid followed closely the seasonal changes in tissue weight with a rise from a minimum value in the winter coinciding with the increase in tissue weight in the spring to give maximum values in sexually ripe animals. The cessation of reproductive activity was followed by a corresponding decrease in the lipid content. Percentage carbohydrate values were highest in the autumn and declined, although with considerable fluctuation to a seasonal minimum coinciding with the period of maximum gonadal proliferation in April–May. Percentage nitrogen values fluctuated in line with these changes in lipid and carbohydrate, reaching maximum values during the winter and minimum values in the autumn. The ash content remained fairly steady at 18–24% except for a single high value in October, 1973.

As is commonly the case in studies of biochemical composition (Ansell, 1974b), the values for lipid, carbohydrate, ash, and protein (taken as 6.25 × %N), when summed often give a total of less than 100%. In the present case the values gave a mean total of 94.4% which compares with a mean total for *D. vittatus* (Ansell, 1972) of 92.5%. The difference is not significant, and following the same practice as in the earlier paper, no adjustment was made of this lack of summation to 100% in calculating weights for a standard animal or calorific values.

## COMPOSITION OF A STANDARD ANIMAL

When the changes in tissue weight and biochemical composition are combined to show the seasonal changes in composition of a standard animal (Fig. 5), it is clear that the reproductive period is marked by an increase in nitrogen (protein) and lipid content, compared with the inactive period, with maximum values for these components maintained between May and August. With the exception of a single high value for carbohydrate in September 1971 (not repeated in 1972) the carbohydrate content of a standard animal remained more or less constant throughout. Ash content rose steadily from the winter of 1971–72 to a maximum in August 1972, and then fell.

## CALORIFIC CONTENT

Following previous practice (Ansell, 1972), the biochemical data have been converted by application of the calorific factors; 5.7 for protein (N × 6.25), 9.5 for lipid, and 4.2 for carbohydrate, to give values for the calorific content of the tissues of *D. trunculus* and for the seasonal changes in total calorific content of a standard animal (Fig. 6). This allows a direct comparison with the earlier study of *D. vittatus*, although by using these factors the calorific content may be over-estimated, since Craig *et al.* (1978) and Beukema & De Bruin (1979) have shown that calorific con-

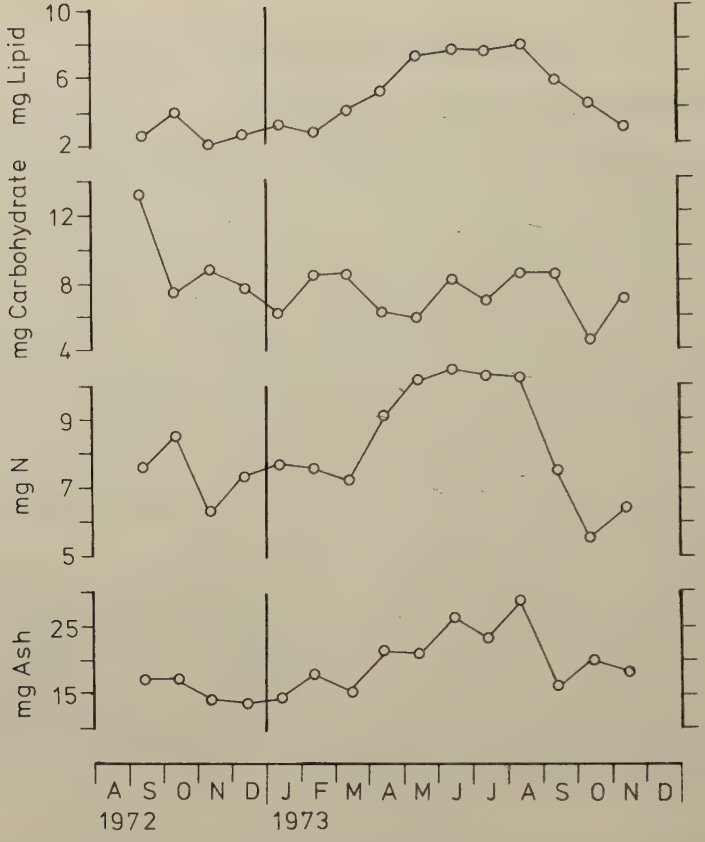


Fig. 5. *Donax trunculus*: changes in biochemical composition of a standard animal of 2.5 g total weight from the population at Azur Plage, Algeria.

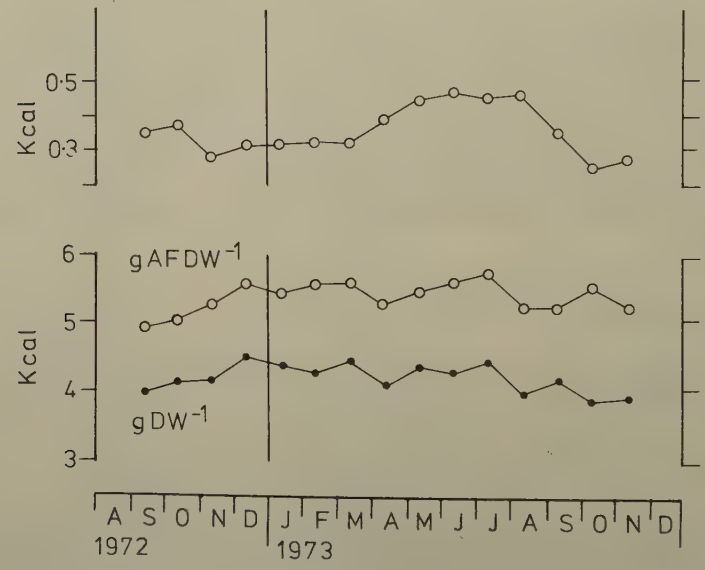


Fig. 6. *Donax trunculus*: calorific content of the tissues (kcal/g dry weight and kcal/g ash-free dry weight) and the calorific content of a standard animal of 2.5 g total weight from the population at Azur Plage, Algeria.

version factors for lipids are often lower than 9.5 cal/mg. The total calorific content varied between 3.9 and 4.5 kcal/g dry weight (4.9 to 5.8 kcal/g ash-free dry weight) with little indication of a clear seasonal change. The calorific content of a standard animal was lowest in the winter months, increasing between March and May to a maintained high level for May to August, when it then fell rapidly.

## Discussion

The results given here for *D. trunculus* on the North African coast confirm and extend observations on seasonal changes in this species, and on the related European species, *D. vittatus*, at locations on European coasts. Ansell (1972) has described the seasonal cycles for *D. vittatus* in Kames Bay, Millport, Scotland, and Bodoy & Massé (1979) and Ansell & Bodoy (1979) have described preliminary observations on a Mediterranean population of *D. trunculus* at They de la Gracieuse on the Camargue coast of southern France. In addition, Badino & Marchionni (1972) recorded some observations on the gonadal cycle of *D. trunculus* from Leghorn (Italy) and Poli (1972) described some features of the reproductive cycle of *D. trunculus* in the Arcachon Basin, on the French Atlantic coast. It is of interest to compare the main features of the cycle on the north African coast with these other studies.

*D. trunculus* at Azur Plage shows a distinct seasonal cycle in tissue weight with associated changes in biochemical composition, which is, moreover, closely related to the seasonal reproductive cycle. The main features of this cycle closely resemble those described by Ansell (1972) for *D. vittatus*, which are also found in other northern-boreal shallow-water bivalves (Ansell & Trevallion, 1967; Hancock & Franklin, 1972; Beukema & De Bruin, 1977). Thus, there is a short period of inactivity during the winter when reproductive activity is minimal and when the sexes are not distinguishable macroscopically. Rapid development and proliferation of the gonad accompanied by a rapid increase in tissue weight follows renewal of activity during the spring. There is an extended reproductive period during the summer when body and shell growth, gametogenesis and spawning may all proceed together; this period is brought to an end in the autumn by the cessation of gonadal recovery marking the return to the inactive period.

In some features the details of the cycle differ from that found for *D. vittatus*. Notably, the changes in tissue weight and in composition are less extreme, reflecting the lesser importance in the cycle for *D. trunculus* on the north African coast of the seasonal accumulation of large metabolic reserves which are later utilized during prolonged periods of environmental stress, when compared with *D. vittatus* from Scottish waters.

During the period of study the dry tissue content of a standard animal showed a maximum rise within any year of 1.8 times the seasonal minimum. For *D. vittatus* (Ansell, 1972) the comparable increase was 2.3 times. The maximum variation in carbohydrate content of a standard animal for *D. trunculus* was about

threefold, while for *D. vittatus* there was a fivefold variation, with a consistent cyclical pattern which was lacking in *D. trunculus*: in *D. vittatus* the carbohydrate content increased fivefold in spring, while *D. trunculus* showed a negligible increase in the same season and in *D. vittatus* there was a similar increase prior to the winter which was absent in *D. trunculus*. The lipid content of a standard animal also showed a seasonal fivefold variation in *D. vittatus* compared with only a twofold variation in *D. trunculus*. *D. trunculus*, at Azur Plage, does not show those features of the seasonal cycle which are particularly associated with storage and utilization nearly as markedly as northern-boreal species. In this the results here confirm those found by Ansell & Bodoy (1979) for *D. trunculus* on the Camargue coast of France.

The values for percentage total lipid found here during the autumn and winter are similar to those found, for the same period, by Ando *et al.* (1976). They showed that total lipid (measured by Soxhlet extraction) fell from 7.5% of dry tissue weight in September to 4.7% in January. The greatest increase in the total lipid content is associated with the period of vitellogenesis.

The seasonal cycle for *D. trunculus* at Azur Plage differs from that described by Ansell & Bodoy (1979) for the Camargue coast, however, in some details, which can best be described by comparison of the cycle in dry weight condition index. The range of values found seasonally for this index was similar in the two areas, but in the Camargue population, in 1977, the values rose more quickly to maximum levels in the spring, while in the autumn, although there was a fall in August and early September this was followed by a renewed increase, not associated with any renewed gonadal activity, so that throughout the winter of 1977–78 the condition index was maintained at fairly high levels when compared with the Azur Plage population. These differences can only reflect differences in the seasonal availability of phytoplankton, and hence productivity in the two areas, and of temperature; the Camargue population is situated within the influence of the nutrient-rich waters of the Rhone estuary, while the Azur Plage population is in an area affected by Atlantic waters (Bernard, 1951; Bernard & Bertoldo, 1958). Only more detailed studies of the seasonal fluctuation in phytoplankton biomass available as food throughout the seasonal cycle than are at present available for the two areas will resolve such differences.

Finally, it is interesting to note the similarity in the seasonal variation of the reproductive cycle from those points in the geographical range where it has been studied. It is clear from the description of Moueza & Frenkiel-Renault (1973), Badino & Marchionni (1972) and Ansell & Bodoy (1979) that the main features of the cycle, and the timing, are similar in these three Mediterranean populations. The observations of Poli (1972) for a population in the Arcachon Basin on the French Atlantic coast show a similar cycle in this area. For more northerly populations the only information available is a statement by Lucas (1965) who studied the cycle of reproduction of *D. trunculus* at Morgat, Nr. Brest. He states "La saison de reproduc-

tion est relativement courte, avec maturité maximale en mai–juin. L'aspect du cycle rapelle celui de *Scrobicularia plana*". The cycle shown by Lucas for *S. plana* shows the number of ripe individuals rising from March to a maximum in June and declining in August–September. In May, one of us (A.D.A.) collected a sample of adult *D. trunculus* at Pentrez Plage, near Morgat, and in all these animals the sexes were clearly distinguishąble. The mean dry weight condition index was 0.927, i.e., higher than the maximum values reached for Azur Plage (this paper) or the Camargue coast (Ansell & Bodoy, 1979). The available evidence, therefore, suggests that the reproduction period is very similar throughout the range of this species.

## Acknowledgements

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